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THE SOIL SOLUTION AND ITS IMPORTANCE IN THE GROWTH OF PLANTS[1]

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The complex relationships of soil, climate and specificity of the nature of the cultivated plants lead to varying yearly crop yields. To be able to differentiate the yearly relationship between the soil, weather conditions and plant, the individual factors influencing the growth of the plants have to be differentiated and their importance determined.

Agriculture is always determined in arid regions by that quantity of atmospheric precipitation, which occurs during the period of plant growth and the period of soil preparation previous to the seeding. However, the quantity of precipitation during the whole vegetation period does not yet determine the quantity of the yield—the amount of precipitation is particularly important at definite periods of the growth of plants, while at other periods it is either of little use or entirely useless.

A larger or smaller quantity of rainfall increases the quantity of soil moisture and there is a corresponding change in the concentration of the soil solution; this in its turn, influences the entrance of the dissolved nutrients into the plant roots. Investigations of the importance of osmotic pressure of the soil solution upon the growth and yield of spring wheat were begun by the author in 1910 at the Bezentschuk Experiment Station, in the state of Samara. These investigations are still being continued and are devoted, not to the development and yield of wheat, but to its chemical composition and protein content.

The original experiments on various salts of the same concentration convinced us at an early date that the same concentration of the soil solution produced by various salts, and therefore of different osmotic pressure, does not act alike upon the growth of plants. At first we followed the swelling and the germination of the grain and confirmed the results of earlier workers, viz., Buffum, and Slosson at the Wyoming Experiment Station. We then began to investigate the growth of plants in pots, in extensive experiments at the Bezentschuk Experiment Station during 1910–1916, then in Petrograd during 1917–1920, and finally in Saratov since 1921.

The experiments were conducted with some local soil, a black soil (tschernoziem) in Bezentschuk and Saratov and a clay soil in Petrograd, in ordinary Wagner pots, 20 cm. in diameter and 20 cm. high. To 5 kgm. of absolutely dry soil, all the minerals were usually added which would favor the maximum

[1] This is merely a summary of extensive investigations on osmosis. The detailed studies will be published later.

crop-yield. The osmotic pressure of the soil solution of a normal pot containing the soil, nutrients and 60 per cent of the moisture-holding capacity renewed daily, was taken as a unit of comparison; this was increased in other vessels by the addition of common salts, NaCl, Na_2SO_4, $MgCl_2$, $NaNO_3$, $(NH_4)_2SO_4$, NH_4NO_3, etc., in such quantities as to increase, when in water solution, the osmotic pressure by 1, 2, 3 atmospheres, etc. These salts were usually added in full amounts when filling the pots with the soil, and sometimes the addition of salts was extended during the whole period of growth of the wheat. At first, wheat seed, *Belaturka* (*Tr. V. hordeiforme*) was taken from a general lot of seed, but later a purer strain was selected at the corresponding Experiment Stations.

Growth, yield, hygroscopic water, total nitrogen and protein and later the hardness and softness of the grain when cut were determined.

INFLUENCE OF OSMOTIC PRESSURE ON THE GROWTH OF WHEAT

An increase in osmotic pressure of the soil solution arrested to a marked degree the sprouting of the seed and weakened it considerably. It seems that the more concentrated solutions enter the seed much more slowly and germination begins later. All the following phases of growth were also arrested; after the period of blossoming was over and ears were formed, the period of ripening was considerably hastened and the total period of vegetative growth for plants upon soil with an increased osmotic pressure was usually markedly less than in normal soils, usually by 6–7 days. In the presence of nitrates the plants behaved differently, since the nitrates, as is well known, extend appreciably the vegetative period of the growth of wheat. Although an increased osmotic pressure of the soil solution arrested the growth of the plants, it is important to note that, with a certain optimum osmotic pressure, the development of the plants reached its maximum. The growth of wheat plants obtained in 1914 and 1915 is given in table 1.

Table 2 gives the influence of various osmotic pressures upon the yield of tops. The data are given for the average of the salts grouped according to their osmotic pressures.

These data definitely established the fact that, with an increase in osmotic pressure of the soil solution, there is an increase in the yield of tops until a certain optimum is reached; on further increasing the osmotic concentration of the soil solution above certain limits there is a decrease in yield. The yield of wheat grain per vessel in relation to the osmotic pressure is given in table 3, which agrees closely with table 2. The data are averages of the results obtained for the same period for all the salts.

From numerous experiments with various salts, it can be concluded that an increase in osmotic pressure of the soil solution up to a certain limit, caused by introducing nutritive salts into the soil, will bring about a greater activity of the plants; under certain conditions there will be an increase in size, yield

of tops and yield of grain of spring wheat. Further increase of osmotic pressure decreases the yield of tops and grain.

As stated above, the wheat grain was analyzed, with special emphasis laid upon the nitrogen content as characterizing the amount of protein in the grain, and, therefore, the quality of the grain itself. A great deal of information has been obtained on this subject, which definitely establishes the character of the influence of an increased osmotic pressure of the soil solution upon the nitrogen and protein content of wheat grain. Since all the data on the nitrogen content of wheat grain grown under different osmotic pressures of the soil solution cannot be presented here, we can only give the averages for different salts and for different years, the data for most salts being for the years 1912 and 1915.

TABLE 1

The average height of wheat in pots for two years

YEAR	NORMAL	1 ATM.	2 ATM.	3 ATM.	5 ATM.	7 ATM.
	cm.	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*
1914	113.2	126.5	116.6	115.5	103.6	84.0
1915	120.2	132.8	125.2	111.6	83.9	72.6

TABLE 2

Average weight of tops per pot in 1914–15

NORMAL CULTURE	1 ATM.	2 ATM.	3 ATM.	5 ATM.	7 ATM.
gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
54	56.9	57.6	52.3	35.8	18.2

TABLE 3

Average yield of grain per vessel

NORMAL	1 ATM.	2 ATM.	3 ATM.	5 ATM.	7 ATM.
gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
20.2	22.2	22.3	20.3	13.2	6.3

Table 4 definitely establishes the fact of increase of total nitrogen and protein nitrogen in particular in the grain of wheat by an increase in the osmotic pressure of the soil solution.

The results of these determinations clearly indicate that the increased content of nitrogen and protein in the wheat grain of southeastern Russia and the arid parts of the United States is doubtlessly connected with the increase in osmotic concentration in the soils of these sections because of the comparatively small amount of rainfall in those sections during the growth of wheat. Table 5 shows the nitrogen content of the grain calculated from tables 3 and 4 and expressed in terms of the normal pot as 100. This indicates that an increase in osmotic pressure caused an increase of the

total nitrogenous substances in the grain of wheat. Even where the total yield of grain was comparatively small, its nitrogen content was such that the total nitrogen yield was greater than in normal pots. As in all the previous cases, the increase to 2 atmospheres osmotic pressure was most favorable; not only was the growth of wheat best in this case, but also the greatest nitrogen yield was obtained per field plot or pot.

It was noted that the grain of the same wheat in the pot studies varied in quality as different quantities of salts were added. In the pots with a normal soil solution there was a large quantity of soft grains together with purely hard grains; in the pots with an increased osmotic pressure, the wheat grains were always all hard. Table 6 shows results obtained with sodium sulfate in 1915. It is thus clear that the quantity of hard grain is increased with an increase in osmotic pressure and *vice versa*, with a comparatively low osmotic pressure of the soil solution, the grain of the same wheat is almost fully soft.

This information allows us to believe that, in vegetative experiments, the same variety of wheat can be made to produce both hard and soft grain.

TABLE 4

Nitrogen content of the grain, Belaturka wheat

	NORMAL POT	1 ATM.	2 ATM.	3 ATM.	5 ATM.
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Total nitrogen.....	1.758	2.076	2.342	2.520	2.773
Protein nitrogen.....	1.647	2.014	2.247	2.518	.....

TABLE 5

Relative nitrogen content of grain

NORMAL POT	1 ATM.	2 ATM.	3 ATM.	5 ATM.
100	129.8	146.3	143.0	109.2

The experiments of 1915 were continued the following years including 1922, and the information obtained in this period of time fully confirms this conclusion. We also noted that by introducing various salts into the soil, we could influence still further the character of softness and hardness of the grain. It was found that nitrates bring about greater increase in hardness than sulfates and that, in addition to the influence of the osmotic pressure of the soil solution, one has to note also the nature of its constituent salts.

Numerous data which have accumulated during the last 12 years indicate rather clearly that the osmotic pressure of the soil solution influences the transpiration coefficient of the plants studied. Table 7 shows the relationships observed in the experiments of 1914 and 1915 as averages for all salts used. Increasing the osmotic pressure of the soil solution, decreased the quantity of water required to form 1 gm. of crop yield. The plants, therefore, used the soil moisture more economically with an increase in the osmotic pressure of the soil solution.

In the last few years, these investigations have been extended to include other plants. The chief object has been to find out whether an increase in osmotic pressure of the soil solution has an influence upon the formation of other substances, such as fats, sugars, aromatic bodies, etc. These experi-

TABLE 6

Hardness and softness of wheat grain at different osmotic pressures

OSMOTIC PRESSURE	SOFT GRAIN	HARD-SOFT GRAIN	COMPLETELY HARD GRAIN
atm.	*per cent*	*per cent*	*per cent*
0.5	27.8	70.0	2.2
3.0	3.6	83.0	13.4
5.0	1.7	48.0	50.3
7.0	0	0	100.0

TABLE 7

Transpiration coefficients with different osmotic pressure

NORMAL CULTURE	1 ATM.	2 ATM.	3 ATM.	4 ATM.
365.6	348.6	333.3	329.9	314.8

ments have not given as yet definite results, since they could not be carried out as completely as necessary, but the results obtained suggest several hypotheses. The work is being carried out with oil-containing plants (flax and mustard) and aromatic plants (mints) and is planned for sugar beets.

ON THE QUESTION OF OBTAINING THE SOIL SOLUTION

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The moisture and nutrition factors of southeastern Russia play an important part both in the life of plants as well as in the soil-forming processes. The Department of Soils, in studying the questions of the life of the soil, has, therefore, taken up the study of the dynamics of the soil solution as one of the principal factors of plant growth and for the purpose of throwing light upon the genesis of local soils

In carrying out the work outlined, the usual methods of obtaining the soil solution were of little value. The most common method of obtaining the soil solution, that of Morgan, consisting in mixing the soil with petroleum oil, pressing and centrifuging, can give, for the dry soils of the Southeast, only a small quantity of soil solution, with which it is very difficult to operate in physico-chemical analysis. By means of the method of L. I. Briggs and I. W. Mehane based on the action of centrifugal force, only a small quantity of the soil solution is obtained even with a comparatively high moisture-content of the soil. The method of Ischtscherekov, consisting in pressing out the soil solution by means of alcohol, has the same disadvantages as the previous methods. Other methods could not be utilized in the laboratory due to technical difficulties through which the country was passing.

These considerations led to the necessity of developing a special method for obtaining the soil solution in a comparatively large quantity more quickly. An apparatus was constructed for this purpose which is based upon the evacuation of the atmosphere within a hollow cylinder, placed within a definite volume of soil. The pressure of the outer atmosphere displaces the water from the soil into this cylinder, as soon as sufficient vacuum has been produced. To obtain sufficient soil solution with a moisture-content of less than 50 per cent saturation and to counteract the molecular forces in the soil, which tend to hold small quantities of moisture, the soil has been pressed by means of a common press in addition to the air evacuation. The apparatus is shown in figure 1. It consists of combination of a Karting suction pump *A* of which the high-vacuum cylinder *B* is a part, press *C*, hollow plates *D*, metallic cup *F*, potassium tube *E*, and vessel *G*.

Cylinder *B* consists of a metallic netting of 8-mm. mesh fused where wires cross to give added strength.

A stopper with a tight fitting glass tube is fitted into the upper part of

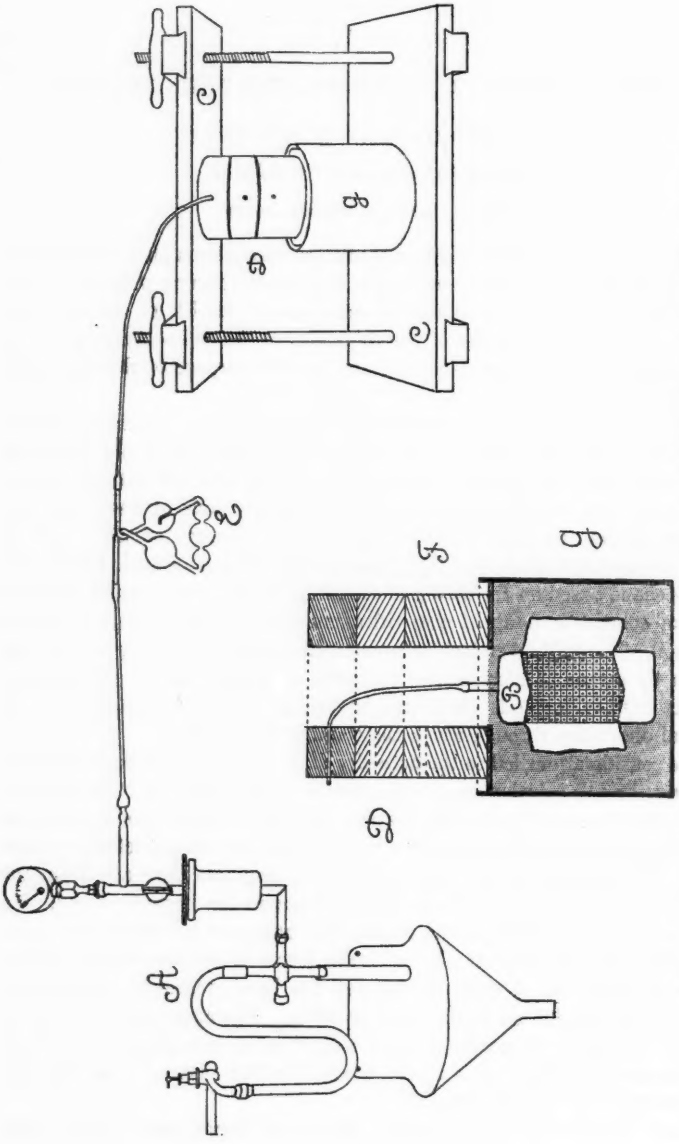

FIG. 1. DIAGRAM OF APPARATUS FOR EXTRACTION OF SOIL SOLUTION BY ATMOSPHERIC PRESSURE.

White flaps represent batiste, turned back

the cylinder. The net is then paraffined, which reduces the mesh to 3–5 mm. in diameter. It is then tightly covered with batiste to exclude soil particles. The volume of the cylinder varies from 10 to 150 cc., depending on the quantity of soil from which the soil solution is to be extracted. The cylinder is connected with the suction pump by means of rubber tubing through the potassium, tube E which serves as an indicator of the action of the pump and the whole apparatus.

The compacted soil adhering to the outside of the cylinder prevents the return of moisture to the soil.[1]

In adapting the press to the apparatus, a metallic plate *F* is placed upon the surface of the soil in the vessel *G;* the plate is then connected by a system of cylindrical plates *D* with the upper cover of the press. The metalic plate and the vessel are covered inside with paraffin.

In obtaining the soil solution, it is important to see that the rubber tubes connecting the parts of the apparatus fit well and also that the soil adheres well to the cylinder so that a vacuum is formed when the pump is working. The last factor is particularly important when the moisture-content of the soil is low. The soil is, therefore, compressed slightly by means of the press preliminary to the action of the suction pump. The pump is then started and as the volume of the soil in the vessel is diminished, pressure is gradually applied to the soil. The first drops appearing in the potassium tube indicate that the cylinder is filled with the soil solution which is then taken out by means of a siphon placed within the cylinder. The soil solution thus obtained is usually slightly turbid and opalescent, particularly the first portions. It is, therefore, filtered through filter paper immediately. It takes 4 to 30 minutes to extract the soil solution by the above method, depending on the moisture-content of the soil. With a moisture-content greater than 25 per cent of the weight of absolutely dry soil, 200 cc. of soil solution is obtained in 10–15 minutes.

Extracts of the cultivated portion of a dark-brown soil were made by this method. Until the present, soil solutions were obtained from this soil by the use of suction and pressure with a moisture-content of 17 per cent of the absolutely dry weight. To characterize the chemical and physical properties of the soil solution obtained by this method we may cite the data obtained from experiment 17. The soil was taken from a portion of the fallow land of the experimental field of the station; the moisture was brought to 38.7 per cent of the absolutely dry soil, corresponding to 90 per cent of the total moisture-holding capacity, and kept at that moisture for 13 days. The soil solution was then extracted. To determine the uniformity of the soil solution, it was taken out in 15 separate 60-cc. portions. A total of 894 cc. of soil solution was obtained from a total of 2250 cc. present in all the soil.

[1] This apparatus has been modified by substituting for the metallic net a copper cylinder perforated with 3–4 mm. holes. This cylinder is not paraffined but is covered with batiste. Instead of using a rubber stopper and glass tube, connection with the potassium tube *E* is obtained by extending the upper part of the cyljnder into a tube of the same copper over which the rubber tubing is fitted.

After the extraction was completed, soil samples were taken from the different parts of the vessel, for moisture determinations which latter varied between 6.9 and 17.4 per cent of the absolutely dry weight of soil. The results of the analysis of the consecutively extracted portions of the soil solution are given in the tables 1 and 2.

The figures obtained definitely demonstrate the constancy of the concentration of the soil solution. The determination of phosphoric acid in the soil solution did not present any difficulties due to the high concentration of the latter in comparison with a water extract of soils. The difference in the concentration of the solution obtained by the method described here in comparison with a water extract is well illustrated in the data from experiment No. 4. By determining the nitrates in the soil sampled the February 10, 1922, by

TABLE 1

Osmotic pressure and dry matter in the soil solution

NUMBER OF PORTIONS OF THE SOIL SOLUTION	OSMOTIC PRESSURE	DRY MATTER ON THE BASIS OF 1 KGM. OF ABSOLUTELY DRY SOIL
	atmospheres	*gm.*
1 and 2	0.54	0.55
6	0.49	0.50
10	0.51	0.52
12	0.51	0.52
15	0.50	....

TABLE 2

Nitrates, phosphates and organic matter in the soil solution

NUMBER OF PORTIONS OF THE SOIL SOLUTION	NITRATES PER 1 KGM. OF DRY SOIL	P_2O_5 PER 1 KGM. OF DRY SOIL	ORGANIC MATTER PER 1 KGM. OF DRY SOIL
	mgm.	*mgm.*	*mgm.*
1, 2 and 3	11.78	0.71	16.80
5, 6 and 7	11.69	0.73	15.68
10, 11 and 12	11.87	0.73	15.98

the method of extraction with water, 2.1 mgm. were found per kilogram of absolutely dry soil. By changing the moisture-content of the soil to 80 per cent of the total moisture-holding capacity, mixing the soil with the water for 3 minutes, as in the case of preparing the water extract, then extracting the soil solution by the above method which consumed 2 minutes, 30 mgm. NO_3 were found per kilogram of absolutely dry soil. On repeating the experiment with soil samples taken April 11, analogous results were obtained.

Since it was possible now to obtain the soil solution in comparatively large quantities, experiments were carried out on the growth of plants in the solution by the method of water cultures. Wheat, oats, barley, peas, etc. were grown in the green house, to full maturity on a soil solution taken from a field soil with the natural moisture-content. By the use of this method, it is now possible

to determine the osmotic pressure and chemistry of the soil solution in the soil of pot cultures in the different periods of growth of cultivated plants.

It should be noted that the work outlined on the method of obtaining the soil solution is just begun. The data obtained, however, allow us to draw the following conclusions:

1. This method permits extraction from varying quantities of soil, within the limits of 100 gm. and 6–7 kgm.

2. There is more of the soil solution obtained by this method than by the other methods.

3. Comparatively little time is consumed for obtaining the soil solution by the method described.

4. The soil solution obtained is of constant concentration in the consecutive portions.

5. By changing the ratio of water to soil more concentrated solutions are obtained for chemical analysis than by water extracts of the soil.

MICROBIOLOGICAL ANALYSIS OF SOILS AS AN INDEX OF SOIL FERTILITY: V. METHODS FOR THE STUDY OF NITRIFICATION[1]

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INTRODUCTORY

In the study of any soil bacteriological phenomenon, we must differentiate between the activities of the microörganisms themselves and the influence of the chemical condition of the soil upon them. From a practical point of view, however, particularly when a correlation is sought between the bacteriological function and crop productivity, a fine differentiation between the bacteriological and chemical soil condition could hardly be expected and may be entirely unnecessary.

When equal amounts of soil are introduced into sterile media of the proper composition and the transformations taking place are studied, we may be able to differentiate between the relative abundance of specific bacteria and perhaps also point out differences in fertility. The information thus obtained will not, however, tell the whole story: a physiological group of microörganisms may be represented in two soils by equal numbers, which may be of different efficiency. If conditions are favorable (selective) for the activities of one particular group of microörganisms and there is a long incubation period, the finer differences between activities of this particular group in the various soils will tend to be obliterated. This is true both of the original Remy method (38) and the modification suggested by Löhnis (31, 32), whereby soil extract is used in the medium.

The use of fresh soil, to which one ingredient is added and the change of which is studied, would seem to present a more natural condition than the solution method. But here again, we must carefully consider the transformation that we are studying. As pointed out by Meyerhof (35), nitrification is at an optimum when the reaction is distinctly alkaline; with an increase in acidity, nitrification rapidly decreases. When we compare, therefore, the

[1] Paper No. 100 of the Journal Series, New Jersey Agricultural Experiment Stations, Department of Soil Chemistry and Bacteriology.

This paper will appear in RUTGERS COLLEGE STUDIES, vol. 1.

[2] The author wishes here to express his thanks to Miss Clara H. Wark for her faithful assistance in the laboratory work involved in these experiments.

nitrifying capacity of two soils, one more acid than the other, or one unlimed and the other limed, the former is bound to give a lower nitrifying capacity using ammonium sulfate or a high grade organic nitrogenous material even though it may be a more fertile soil. When we compare the nitrifying capacity of two soils by the common method of adding ammonium sulfate (much in excess of what we would add to field soils) to 100 gm. of soil and determining the nitrates formed after incubation, we actually measure not the nitrifying capacities of the two soils but merely, in a roundabout way, the initial reaction, buffer content, and presence of neutralizing substances of the soil. The latter could be done, within a shorter period of time and more accurately by chemical methods. Dried blood has found favor with some bacteriologists as a substratum for the study of nitrification; this is, no doubt, due to the fact that the ammonia rapidly produced from dried blood in acid soils tends to make the reaction of the soil less acid and, ipso facto, make conditions more favorable for an abundant accumulation of nitrates.

In studying a bacteriological function, such as nitrification, for the differentiation of soil fertility we must, therefore, make certain that it is only the function in question that is studied, including the chemical conditions of the soil (such as stimulants, etc.) influencing it. When we use it, however, as an index of a certain chemical condition of the soil, such as soil reaction, abundance of lime or available phosphates, we must definitely differentiate between this information and the general consideration of soil fertility.

HISTORICAL

For the study of nitrification in soil, Remy (38) used at first two solutions, one with ammonium salt as a base and one with nitrite. Löhnis (31, 32) limited himself to one solution containing only 0.1 per cent ammonium sulfate; 5 gm. of soil or soil suspension were inoculated into 50 cc. of medium and incubated 30 days at 20°C. and then the nitrates were determined quantitatively. Barthel (5) suggested aeration of the flasks for 20 days at 20°C.

The use of liquid media for the study of nitrification has met with a great deal of criticism and has been found by some investigators to be much less favorable than the use of soils, since nitrifying bacteria are more sensitive to organic substances in solution than in soil. Stevens and Withers (41, 42) have shown that soil organisms nitrify less in liquid than in soil, while bacteria from sewage purification act better in liquid media. No relation was found between bacterial activities in soil and in a suspension of the same soil. Tests in solution were found inadequate for indicating the nitrifying power of a soil, and soil itself was recommended as a medium for the study of nitrification. Similar observations as to the study of nitrification in liquid media were made by Koch and Petit (27), Koch (26), Fischer (10), Heinze (19), Lemmermann and associates (28), and Vogel (45). Kellerman and Allen (22), using the soil method, obtained not only a close correlation between nitrification and soil productivity, but even a definite differentiation between soil samples taken at different depths. By the use of the solution method, however, no differentiation was obtained between a good and a poor soil and, in some cases, samples taken from the lower depths allowed as much nitrate accumulation as the surface samples. Nitrification of organic materials cannot be studied in liquid media due to the toxic effect of the soluble organic compounds and the ammonia formed. Kellerman and Robinson (23) further state definitely that nitrifying bacteria do not act normally in test solutions.

Löhnis (33), Löhnis and Green (34) and Green (17) discussed the subject in detail in later contributions and found that, for the study of nitrification, solution methods are quite as valuable as methods in which the natural soil is used as a medium. They very properly pointed out that a low lime content may act as a limiting factor to nitrification in soil tests, when ammonium sulfate is used, although not in the field, so that the method of study of nitrification in soil may be merely an index of lime content rather than of nitrifying efficiency. In the field, where only relatively minute quantities of ammonia are to be nitrified, the low natural lime content need not enter as a limiting factor.

A correlation between soil type and nitrification by the solution and soil method was found by Fischer (10), but the results obtained by the latter method were more pronounced. A definite correlation was found by Wohltmann, Fischer and Schneider (51) between soil fertilization and nitrification (lime was found to have a particularly favorable effect on nitrification of ammonium sulfate) in the soil as well as in solution, but the differences found by the latter method disappear on prolonged incubation.

The solution method for the study of nitrification gives, however, information that could not possibly be obtained by the soil method alone, such as the distribution of the organisms noted by Allen and Bonazzi (2), activities of the organisms not in their natural substrata, as well as in very adsorptive moor soils mentioned by Ritter (39). If the results obtained by the solution method do not give a direct absolute comparison with soil productivity, the relative comparison may be of great importance in supplementing the information obtained by the soil method.

The soil method, as commonly carried out, consists of adding a certain amount of an inorganic salt of ammonium, usually the sulfate, 0.1 to 1.0 per cent, or organic nitrogenous material, usually dried blood, at the rate of 0.5 to 2.0 per cent to 1–100 gm. of soil. The soil is kept at optimum moisture for 10, 20 or 30 days, at a temperature of 25 to 28°C. The nitrates formed are determined either by the phenol-di-sulfonic or by reduction methods.

Temple (43, 44) found that various nitrogenous organic substances and ammonium salts of organic acids nitrified in the soil faster than the ammonium sulfate and chloride. This was not due to the nature of the nitrifying organisms in the soil, since pure cultures of nitrifying organisms obtained from a number of sources produced the same result. When $CaCO_3$ was added to the soil, ammonium sulfate nitrified as well as any of the other substances. These soils (of the Cecil group) were all acid; the soil organisms decomposed the substances of organic origin in a way that more ammonia than acid was produced thus correcting the acidity and bringing about a condition favorable for the growth of the nitrifying organisms. With ammonium sulfate and chloride, the soils became even more acid due to the liberation of the anion and transformation of cation into nitric acid. When lime was added to the soil, the relation was reversed.

Fred and Graul (12) also found that, in acid soils, organic nitrogen nitrifies much quicker than ammonium sulfate, regardless of the source of nitrifying bacteria. In neutral or alkaline soils the reverse takes place. Barthel (6) suggested that the poor nitrification of ammonium sulfate in acid soils is due to the fact that, as soon as nitrification begins, nitric and sulfuric acid accumulate in the soil and reduce the hydrogen-ion concentration of the soil to a degree, which checks the further development of the nitrifying bacteria. But, when lime is added, the acid is neutralized, and no increase in the hydrogen-ion concentration of the soil will take place.

The lack of nitrification in a certain soil need not necessarily indicate the absence of nitrifying organisms, but merely an unfavorable chemical condition of the soil, as shown by Arnd (4) in his study on nitrification in peat soils. Raw, unlimed peat soils, acid in nature, are unable to nitrify the ammonia formed in the soil from the decomposition of organic matter or when added directly to the soil. When enough basic material ($CaCO_3$) is added to neutralize the acid, active nitrification takes place. Low peat soils, of a neutral reaction, need no liming for nitrification. An increase in lime much above the neutral point may result in lower nitrate accumulation.

The use of inorganic salts of ammonia and organic materials has not found equal favor in the hands of the various investigators. Some have favored one; some, the other, as giving a true picture of the nitrifying capacity of the soil. Some have used both methods, without trying to explain the difference in results obtained by the two methods. The peculiar influence of lime on nitrification, both on the oxidation of inorganic and organic materials, has been observed by various workers. No attempt has usually been made (except by Barthel (6) and a few others to correlate this with the original and final soil reaction as well as the influence of ammonia produced from the decomposition of the organic material, under alkaline conditions, on the action of the nitrifying bacteria. Beckwith and associates (7), for example, found active ammonification taking place in six Oregon soils, but in two soils, less nitrate was found after four weeks incubation, both with dried blood and ammonium sulfate, than in the original soil itself. In one case the further addition of lime failed to induce the nitrification of these materials, although the nitrifying bacteria were present.

One is almost certain to find, by going over the literature on nitrification, that ammonium sulfate is not nitrified or only to a small extent in acid soils, particularly soils that are poorly buffered; but it is very vigorously nitrified in neutral and alkaline soils, particularly rich in organic matter. Lipman and Burgess (30) found the latter to be true for California soils, while Temple (44) and others found the former to be true for acid soils.

Dried-blood and high-grade tankage, as well as other high-grade nitrogenous materials will nitrify well in not too acid soils, particularly in well buffered soils, but they will not nitrify readily in alkaline soils, or in soils poorly buffered (with low humus content). Low grade nitrogen materials will nitrify better in soils with a low buffer content, due to the fact that the relatively smaller ammonia formation will not tend to injure the activity of the nitrifying bacteria, while these materials will offer a greater buffering agent to the accumulation of nitric acid. High grade nitrogenous materials, particularly in amounts used for laboratory tests, allow such a rapid and intense accumulation of ammonia that the nitrifying bacteria are injured in their activities. The injurious effect of ammonia on nitrification has been long recognized but, while Winogradsky (50) believed that ammonium sulfate was also toxic, Meyerhof (35) demonstrated that it is free ammonia and not the ammonium salt which injures the nitrate-forming bacteria and that the salt is uninjurious even in solution, in the presence of buffering substances like phosphates. Under alkaline conditions, particularly in the presence of sodium carbonate or bicarbonate, the ammonium salt will interact with the carbonate giving free ammonia. In soils with a low humus content, particularly of a neutral or alkaline reaction, there are not enough acid radicals or buffering materials to combine with the ammonia formed abundantly from the high grade nitrogenous material used for the test, and the latter becomes injurious.

This explains also the observations made that small amounts of K_2CO_3 stimulate nitrification, while larger applications of the salt progressively diminish the rate of nitrification. It explains also the fact that $CaCO_3$ may have a retarding effect on nitrification, particularly of blood-meal, as found by Beckwith and associates (7), or a favorable effect on the nitrification not only of ammonium salts, but organic nitrogen compounds, as shown by Fred and Graul (12) who worked with acid soils.

Kelley (24, 25) found that nitrate formation from dried blood, bone meal, and ammonium sulfate during four weeks incubation varied enormously when different concentrations were employed. With 1 per cent of dried blood, nitrification was feeble or absent in certain soils in which 1 per cent of bone meal and 0.2 to 0.3 per cent of ammonium sulfate underwent active nitrification. When low concentrations of dried blood were employed, such as are used in the field, active nitrification took place in every case. High concentrations of bone meal with a nitrogen content corresponding to that furnished by 1 per cent of dried blood were also toxic to nitrification. It is interesting to note that 0.05 per cent sodium carbonate was distinctly toxic to the nitrification of 1 per cent of dried blood, while as high a concentration as 0.4 per cent sodium carbonate produced no effects on the nitrification of 0.1 per cent dried blood. This bears out the previous discussion. That sodium carbonate is far

more toxic to nitrification of a high concentration of dried blood than sodium sulfate has been pointed out by Lipman (29); 0.1 per cent sodium carbonate was toxic to 0.15 per cent of ammonium sulfate, and markedly stimulating to 0.0625 per cent. Kelley (25), therefore, concluded that nitrification studies, in which high concentrations of nitrogenous materials are added and nitrates determined at a fixed interval of time, are likely to be more misleading than informing. Kelley recognized quite correctly that the intermediate products formed in the process of nitrification may, directly or indirectly, exert much influence upon the oxidation of ammonia. He states elsewhere (24) that $CaCO_3$ exerts almost no effect on the nitrification of dried blood in the soil. This is of course explained by the facts that the injurious influence of high concentration of dried blood is due to an excess of ammonia formation, particularly in poorly buffered soils; that the effect is even more pronounced under alkaline conditions; that the lack of nitrification of ammonium sulfate may be due more to the acid formed and that this is corrected by the application of $CaCO_3$. It is interesting to note that Kelley obtained active nitrification of dried blood in manured plot, which would increase the buffer content of the soil.

The one general criticism that can be applied to the soil method of studying nitrification is that the differences in the rate of nitrification may depend rather on the physical and chemical properties of the soil, than upon the number and activities of the bacteria originally present in the soil, as shown by Gerretsen (16) and others. The study of nitrification should, therefore, be carried out both in solution and in soil to give us a true picture of the phenomenon.

Differences in nitrate formation are not due to size or shape of container, quantity of soil used or depth of column, as long as the soil is loose, as pointed out by Gainey and Metzler (15).

Before taking up the experimental part of the work, it is not out of place to summarize briefly our knowledge of the influence of reaction on the activities of nitrifying bacteria. Various investigators have recorded the fact that the addition of lime to acid soils results practically in all cases in increased nitrifying activities. This is made clear by the fact that the optimum reaction for the activities of the nitrifying bacteria both of the nitrite and nitrate groups lies on the alkaline side of neutrality.

Meyerhof (35), in an exhaustive study on the oxidation of nitrifying bacteria found that the nitrate formers act, in solution, at reactions ranging between pH 5.6 and 10.3 with an optimum at pH 8.3 to 9.3. The optimum for the nitrite bacteria lies at pH = 8.8. Gaarder and Hagem (13) found the optimum for the growth of the nitrate bacteria to be at pH 7.0–7.2, and for nitrite bacteria at pH 7.8, the kind of buffer being of importance. The difference between their results and those of Meyerhof is explained either by the difference in method used, by an actual difference in races of bacteria or by the fact that Meyerhof studied the respiration of the organisms during a short period of time, while Gaarder and Hagem studied the growth of the organisms.

Gerretsen (16) found the acid limit for nitrification (using the solution method) to be at pH 3.9–4.5, depending on the origin of the nitrifying bacteria, those of acid soils being more adapted to acid conditions. The limiting alkaline reaction was found to be at pH 8.9–9.0, the buffer effect of $CaCO_3$ and $Fe(OH)_3$ being important. The microörganisms resist also a greater degree of acidity in the soil than in solution.

When we add an ammonium salt to the soil for testing the nitrifying capacity of that soil, a further increase in acidity results, so that in the case of acid soils, particularly those with a low buffer content, the limiting reaction is soon reached. The amount of nitrate formed may then be limited by this final acidity rather than by the nitrifying activities of the soil as such, as pointed out above. It is interesting to record that soils containing scarcely any nitrate and capable of nitrifying ammonium salts only to a very small extent, usually nitrify

in solution, indicating the presence of nitrifying organisms. It is, therefore, reasonable to expect that the addition of lime to acid soil will result in an increased activity of the soil nitrifying flora, when ammonium salts are used in the test. Unfortunately, in the majority of studies on nitrification in acid soils, merely the lime requirement of the soil is recorded rather than the reaction expressed in terms of hydrogen-ion concentration. The former expressed not so much the actual acidity of the soil as its combining power with lime or buffer content. The greater lime requirement may indicate both a higher acidity, which is injurious to nitrification, or a higher buffer content of the soil, which may allow a greater accumulation of nitrates, or prove beneficial to nitrification as the test is commonly carried out. This method of expressing acidity is, therefore, valueless as far as the test for the nitrifying activities of the soil is concerned. There is no discrepancy between the statements of some of the earlier workers, Warington (48, p. 42–76, 77–94) and Deherain (9) who thought that acid soils inhibited nitrification which takes place only in feebly alkaline media and may go on in soils deficient in lime, and some of the later workers, as White (49), Stephenson (40), who found that nitrification takes place in acid soils. It depends on the degree of acidity and method of measurement.

Hall, Miller and Gimingham (18) found that continuous treatment of a soil with ammonium sulfate will make the soil so acid as to greatly reduce nitrification. The presence of nitrates in the soil indicates that nitrification probably takes place in the presence of small isolated particles of calcium carbonate. Petit (37), Abbot and associates (1), Temple (44) and others soon reported active nitrification in acid or non-basic soils. Noyes and Conner (36) demonstrated that the amounts of nitrate present and the nitrifying power of untreated acid soils varied with the organic matter and total nitrogen rather than with the soil acidity.

According to the results presented by Stephenson (40) lime as such (in the form of carbonate) does not stimulate the activities of the nitrifying bacteria in the soil, as seen from the fact that no increase in nitrification of the soil's own nitrogen took place as a result of the addition of lime. Lime serves merely as a base for neutralizing the acid formed from the oxidation of ammonium sulfate used in carrying out the nitrification test. The nitrifying capacity of the same soil placed in a series of pots and treated with various amounts of $CaCO_3$ was tested by adding 100 mgm. of ammonium sulfate to 100-gm. soil portions in tumblers. There was a progressive increase in the capacity of the soil to accumulate nitrates by increasing the lime treatment to 7 tons, further additions did not result in any appreciable increase. By adding acid to soil, a much greater repression of nitrification was obtained in a sandy than in a loam soil, due to the lower buffer content of the former, which will result in a greater hydrogen-ion concentration, with the same amount of acid than the loam soil. This again points to the absolute futility of testing the soil by the lime requirement methods when studying nitrification.

To point out the importance of the presence of basic materials, like calcium carbonate, for the neutralization of the acids formed in the process of nitrification, the work of Ames (3) may be cited. Ames found that in an acid silt loam, in the absence of $CaCO_3$, dried blood was nitrified to a greater extent than ammonium sulfate, the nitrates formed from the latter being less than in the untreated soil. When $CaCO_3$ was added at the rate of 4000 parts per million, the nitrification of both forms of nitrogen was increased. With 8000 parts of $CaCO_3$, the formation of nitrate nitrogen from ammonium sulfate exceeded the amount produced from dried blood. Calcium phosphate can take the place of the carbonate in supplying a base for nitrification only to a limited extent for the very obvious reason that while the carbonate is brought into solution by the action of the acid at pH 6.0–7.0, which is optimum for the activities of the nitrifying bacteria, the phosphate goes mostly into solution at pH 3.0, much below the acid limit of their activities.

Fischer (10) found that the theoretical amount of lime (200 mgm. of $CaCO_3$) required for the nitrification of ammonium sulfate (132.7 mgm.) was not sufficient to complete nitrification, but about 3.5 times the theoretical amount is required.

EXPERIMENTAL

The same plots that were used for the study of bacterial numbers (46) and ammonification (47) were employed in this study. It is important to remember that 5A and 5B are the plots receiving 16 tons of stable manure per acre and minerals every year; 7A and 7B, no fertilizer at all; 9A, 320 lbs. of $NaNO_3$ per acre and minerals; 11A and 11B, $(NH_4)_2SO_4$ equivalent in nitrogen content to 320 lbs. of $NaNO_3$ and minerals; 4A, 19A and 19B minerals only; and 18A, manure, nitrate and minerals. All the B plots were limed every 5 years at the rate of 2 tons $CaCO_3$ per acre.

TABLE 1

Nitrification of ammonium sulfate in 100 gm. of soil

PLOT NUMBER	TREATMENT*	NITRATE NITROGEN	INCREASE OVER SOIL	N (IN AMMONIUM SULFATE) NITRIFIED
		mgm.	*mgm.*	*per cent*
4A	Soil itself	0.90		
4A	$(NH_4)_2SO_4$*	1.46	0.56	1.9
5A	Soil itself	1.4		
5A	$(NH_4)_2SO_4$	2.67	1.27	4.2
7A	Soil itself	0.7		
7A	$(NH_4)_2SO_4$	0.48	−0.22	0.0
9A	Soil itself	0.88		
9A	$(NH_4)_2SO_4$	3.03	2.15	7.2
11A	Soil itself	1.6		
11A	$(NH_4)_2SO_4$	0.95	−0.55	0.0
18A	Soil itself	0.81		
18A	$(NH_4)_2SO_4$	6.11	5.30	17.7
19A	Soil itself	0.9		
19A	$(NH_4)_2SO_4$	0.81	−0.09	0.0
7B	Soil itself	1.08		
7B	$(NH_4)_2SO_4$	7.54	6.46	21.5
11B	Soil itself	0.45		
11B	$(NH_4)_2SO_4$	4.80	4.35	14.5
19B	Soil itself	0.85		
19B	$(NH_4)_2SO_4$	9.39	8.54	28.47

* $(NH_4)_2SO_4$ treatment consisted of 30 mgm. of nitrogen as ammonium sulfate added to 100 gm. of soil and incubated for 30 days.

Samples were taken to a depth of 6 and $\frac{2}{3}$ inches by means of a borer from 5 to 6 different parts of each plot, brought to the laboratory, well mixed and sieved through a sieve to remove the stones, coarse pebbles and organic matter. The soil was then placed, in 100-gm. portions, in tumblers, to which the proper amount of ammonium sulfate (in solution) or dried blood were added, well mixed and enough water to bring to two-thirds saturation. The tumblers were covered with glass plates and incubated at 27°C. for 30 days, unless otherwise stated. Moisture was added at weekly intervals to keep at optimum. At the end of the incubation period the soil was well mixed,

5 gm. were usually used for determining the hydrogen-ion concentration, by the colorimetric method, 50 gm. were placed in 500 cc. flasks, to which some CaO and distilled water was added and shaken for 10–15 minutes. The extract was then filtered and an aliquot portion evaporated to dryness and nitrates determined by the phenol-di-sulfonic acid method (Davis (8)).

The nitrates were always calculated back to the original 100 gm. of soil used and recorded as milligrams of nitrate nitrogen. In some cases 200 gm. of soil were used. The ammonia was then determined in one-half of the mixed soil by distilling with MgO. Two or three tumblers were usually used for each determination, but only the averages are recorded, due to the fact that the duplicates checked up fairly well.

The two unproductive plots 7A and 11A did not nitrify the ammonium sulfate at all; this is probably due to the high acidity of these plots; the limed plots 7B, 11B and 19B allowed a much greater accumulation of nitrates from

TABLE 2

Nitrification of soil, ammonium sulfate and dried blood

PLOT NUMBER	SOIL ITSELF (100 GM.)		30 MGM. N AS $(NH_4)_2SO_4$ PER 100 GM. SOIL		1 GM. DRIED BLOOD (11.5% N) PER 100 GM. SOIL	
	Final reaction	NO_3-N	Final reaction	NO_3-N	Final reaction	NO_3-N
	pH	*mgm.*	*pH*	*mgm.*	*pH*	*mgm.*
4A	5.3	1.2	5.0	1.9	5.7	11.8
5A	5.3	1.7	4.6	5.5	5.2	24.5
7A	4.8	0.6	4.8	0.5	6.0	11.7
9A	5.4	1.0	5.0	2.5	5.3	15.5
11A	4.0	1.0	3.8	0.4	5.0	12.5
18A	5.4	2.0	4.6	6.4	4.7	28.8
19A	5.2	1.3	4.9	1.2	5.2	13.7
7B	6.0	1.1	4.4	11.3	5.3	13.8
11B	5.4	1.2	4.8	5.0	5.1	20.0
19B	6.3	1.0	5.0	12.5	6.0	12.6

the ammonium sulfate than the corresponding unlimed plots without any reference at all to productivity but depending entirely on the initial reaction and buffer content of the plots. Where soil containing different amounts of lime and of a different reaction and buffer content are compared in their capacity of nitrifying ammonium sulfate, the results are entirely misleading. The accumulation of nitrates from ammonium sulfate depends upon the final reaction of the medium; the less acid the initial reaction and the greater the buffer content of a soil, the larger will be the amount of nitrate accumulated from ammonium sulfate before the final acid reaction is attained. These two factors prevent us from interpreting properly the results obtained from nitrification studies under those conditions. This is made even clearer in the following experiment, where the final reaction is also recorded.

The ammonium sulfate column in table 2 brings out the same differences as those found in table 1. The nitrate is formed from ammonium sulfate

until the reaction reaches a minimum somewhere between pH 4.4 and pH 5.0 and the total amount of nitrate is determined merely by the initial reaction and buffer content of the soil. The largest amounts of nitrate were formed from 7B and 19B, because of their relatively low initial acidity. Both of these plots are relatively unfertile since they received no nitrogen application for the last 15 years. The next greatest nitrification occurred in 5A and 18A, which are very productive plots and have high buffer contents due to the yearly applications of manure. Plots 7A and 11A, the most acid plots, produced even less nitrates than were formed in the test where nitrification of soil's own nitrogen took place.

The results with dried blood are distinctly different. The largest amounts were formed by 5A and 18A, the two manured plots; then come 9A and 11B, the plots receiving sodium nitrate and ammonium sulfate with lime, next in productivity to the manured plots. However, no such sharp differences are obtained with the dried blood, due to the fact that the ammonia formed from the decomposition of the dried blood neutralizes the acidity of the soil (7A and 11A) allowing nitrification to take place. No such lack of nitrification of 1 per cent of dried blood as observed by Kelley is found here, due to the fact that all these plots are sufficiently acid to combine with the ammonia formed so that it cannot injure the action of the nitrifying bacteria, while Kelley worked with less acid soils and perhaps less buffered, where the ammonia from the dried blood would readily become injurious.

One can readily recognize that ammonium sulfate would bring out much greater and more accurate differences between the different soils, if the rapid formation of a maximum acidity could be prevented. Various amounts of CaO were then added to several lots of the different soils, and after the soil was air dry, the nitrogen substances were added, as well as the proper amount of moisture.

Several important observations can be made from the data in table 3. Nitrification of ammonium sulfate is considerably increased by the application of lime, particularly in soil having a reaction of a pH less than 6.0; if an excess of lime in the form of CaO is added so that the reaction of the soil becomes equivalent to pH 8.0–8.2, nitrification of ammonium sulfate is injured, probably due to the toxic effect of free ammonia formed from the interaction of the oxide and sulfate. In the case of dried blood (using 1-per cent concentration), the injurious influence of the original reaction may set in even at pH 7.4, due to the fact that the reaction becomes quickly alkaline as a result of the ammonia formed from the decomposition of the dried blood; when the soil is richly buffered, the injurious influence does not set in as quickly. This was the reason why Lipman, Kelley and others found that dried blood (1 per cent concentration) does not nitrify in arid soils, poor in humus, which are apt to be of an alkaline reaction to start with.

The following experiment (table 4) deals with the same phenomenon, in a more extensive way.

Nitrification of ammonium sulfate in a well buffered soil (5A) of an acid reaction is greatly increased by the addition of CaO. However, in a poorly buffered acid soil (7A), the stimulating effect of CaO on nitrification is not so marked, and it may rapidly depress it altogether when used in excess. The CaO liberates the ammonia from the ammonium sulfate thus exerting a decided toxic effect upon the activities of the nitrifying bacteria. This toxic effect is marked also in well buffered soils (5B), when the reaction is made too alkaline. A similar phenomenon is observed in the nitrification of dried blood. Even as large a concentration as 1 per cent of dried blood is readily nitrified in well buffered soils (5A, 5B) and may even be stimulated by the addition of small amounts of CaO; in poorly buffered soils (7A, 7B), nitrifica-

TABLE 3

Nitrification of ammonium sulfate and dried blood in the soil, with and without CaO

PLOT NUMBER	SOIL ITSELF (100 GM.)			SOIL + CaO*			30 MGM. N AS $(NH_4)_2SO_4$ IN 100 GM. SOIL			$(NH_4)_2SO_4$ + CaO†		
	Initial reaction	NO_3-N	Final reaction	Initial reaction	NO_3-N	Final reaction	Initial reaction	NO_3-N	Final reaction	Initial reaction	NO_3-N	Final reaction
	pH	*mgm.*	*pH*	*pH*	*mgm.*	*pH*	*pH*	*mgm.*	*pH*	*pH*	*mgm.*	*pH*
5A	5.4	2.0	5.4	7.7	3.0	7.4	5.4	3.2	4.6	7.2	16.0	4.4
5B	6.4	2.8	6.2	7.8	1.5	7.6	6.4	12.5	4.6	7.1	9.5	5.4
7A	4.8	0.5	4.8	7.9	0.9	7.9	4.7	0.21	4.2	7.6	1.8	7.2
7B	5.8	0.75	5.8	7.5	1.2	7.4	5.8	5.6	5.0	7.2	10.0	5.6

PLOT NUMBER	$(NH_4)_2SO_4$ + CaO*			1 PER CENT OF DRIED BLOOD			DRIED BLOOD + CaO*		
	Initial reaction	NO_3-N	Final reaction	Initial reaction	NO_3-N	Final reaction	Initial reaction	NO_3-N	Final reaction
	pH	*mgm.*	*pH*	*pH*	*mgm.*	*pH*	*pH*	*mgm.*	*pH*
5A	7.7	34.0	4.5	5.4	29.8	5.2	7.4	40.6	4.8
5B	7.8	14.0	5.6	6.4	20.6	5.8	7.6	25.6	6.6
7A	8.2	Tr.	8.0	4.8	11.2	7.4	7.4	0.8	8.2
7B	7.4	8.8	5.8	5.8	20.6	5.9	7.4	0.5	8.6

* 200 mgm. of CaO were used for 5A and 5B; 300 mgm. for 7A, and 100 mgm. for 7B.
† 100 mgm. of CaO were used for 5A and 5B; 200 mgm. for 7A and 50 mgm. for 7B.

tion of 1 per cent dried blood may or may not proceed rapidly, depending on the rapidity of ammonia formation. If the ammonia formation is at first slow, nitrification of dried blood may set in rapidly even in poorly buffered soil; if ammonia formation is rapid from the first and insufficient buffering agents are present, nitrification will be repressed. The addition of an excess of CaO even to well buffered soil will have the same effect.

The following experiment was carried out in a similar way by the use of a larger number of soils and results are given in table 5.

The previous observations hold true also for all the 11 soils obtained from the variously treated plots. The amount of nitrate formed from ammonium sulfate depends on the initial and final reaction of the soil and buffer content

of the particular soil. Plot 5B gave about 7 times as much nitrate as 5A. This was due entirely to the fact that the initial reaction of the former was pH 6.4 and of the latter only pH 5.4, while the final reaction was about pH 4.6–4.8. Plot 7B produced 12 times as much nitrate as 7A due to the fact that the initial reaction of 7B was pH 6.0 and of 7A only pH 4.8 which is near the maximum acidity. Plot 19B allowed only a little more than a half the ac-

TABLE 4

*Influence of reaction upon nitrification of ammonium sulfate and dried blood**

SOIL TYPE	SOURCE OF NITROGEN	CaO ADDED	INITIAL REACTION	FINAL REACTION	NH_4-N	NO_3-N
		mgm.	*pH*	*pH*	*mgm.*	*mgm.*
		0	5.4	4.7		5.8*
5A	50 mgm. N as $(NH_4)_2SO_4$	100*	7.0	4.6		16.2
		250	7.4	4.6		31.8
		0	4.8	4.7		0.8
7A	50 mgm. N as $(NH_4)_2SO_4$	200	7.4	7.2		1.8
		300	7.8	7.8		Tr.
		500	8.6	8.6		Tr.
		0	6.4	4.6		37.2
5B	50 mgm. N as $(NH_4)_2SO_4$	100	7.1	4.6		43.8
		250	8.0	7.4		5.8
		0	5.4	5.2	29.52*	45.4
5A	1 per cent dried blood	100	7.0	5.8	10.08	56.8
		250	7.6	7.8	48.56	2.6
		0	4.8	6.8	67.6	1.2
7A	1 per cent dried blood	200	7.4	8.2	62.4	0.8
		300	7.8	8.6	61.8	0.0
		0	6.4	5.5	39.4	16.2
5B	1 per cent dried blood	50	7.0	6.2	6.62	31.6
		250	7.8	6.4	9.82	27.0
		0	6.0	5.8	29.2	20.2
7B	1 per cent dried blood	50	7.2	7.8	49.4	0.5
		100	7.8	8.8	43.12	0.0

* Quantities are given per 100 gm. of soil.

cumulation of nitrate in 5B, with approximately the same initial acidity, due to the higher buffer content of the second soil. When enough CaO is added to make the initial acidity approximately pH 7.2–7.4, nitrification of all soils is increased. Under these conditions the influence of the initial reaction upon the nitrifying capacity is eliminated. Plots 5A, 18A and 5B are the most fertile and give the highest amounts of nitrates. Plots 7A, 19A and 11A are the least fertile and give the lowest amounts of nitrates.

However, the use of CaO with ammonium sulfate brings about a rapid increase in the alkalinity of the soil which may often prove injurious to the action of the nitrifying bacteria. Table 6 is representative of the comparative influence of $CaCO_3$ and CaO upon nitrification in the soil.

To be able to interpret nitrification results, it is important to know the course of nitrate formation in the soil as shown in table 7.

In view of the fact that the activities of the nitrifying bacteria are so much affected by the initial and final reaction of the soil used as a medium, all the soils should be brought first, by means of CaO or acid, to a certain reaction optimum for the activities of the organisms, like pH 7.0 to 7.2 and then tested for the nitrifying capacity; or they should receive a definite amount of $CaCO_3$

TABLE 5

Influence of reaction upon the nitrification of ammonium sulfate and dried blood by 100 gm. of various soils

PLOT NUMBER	SOIL ITSELF			50 MGM. N AS $(NH_4)_2SO_4$		50 MGM. N AS $(NH_4)_2SO_4$ + CaO*		1 PER CENT OF DRIED BLOOD			1 PER CENT OF DRIED BLOOD + CaO*		
	Final reaction	NH_3-N	NO_2-N	Final reaction	NO_2-N	Final reaction	NO_2-N	Final reaction	NH_3-N	NO_2-N	Final reaction	NH_3-N	NO_2-N
	pH	*mgm.*	*mgm.*	*pH*	*mgm.*	*pH*	*mgm.*	*pH*	*mgm.*	*mgm.*	*pH*	*mgm.*	*mgm.*
4A	5.6	1.4	2.5	4.8	2.5	4.9	34.2	5.4	38.1	21.6	7.9	56.7	0.8
5A	5.4	0.8	5.5	4.7	5.2	5.1	56.8	5.0	29.7	39.5	6.4	3.2	54.2
7A	4.8	0.3	1.3	4.8	0.8	7.4	0.6	7.0	47.7	0.5	8.2	42.2	0.0
9A	5.8	0.8	2.2	5.0	8.5	6.1	27.2	6.0	32.9	26.8	8.5	44.6	0.0
11A	4.6	0.8	0.8	4.6	0.2	6.2	19.2	6.9	64.1	0.8	7.8	48.3	0.0
18A	5.5	0.8	2.4	4.9	11.2	5.0	52.6	5.0	28.3	45.5	6.2	4.6	40.4
19A	5.4	0.8	1.1	4.9	2.2	6.1	12.4	6.6	47.8	12.8	8.2	39.2	0.0
5B	6.5	1.2	2.8	4.8	37.3	5.0	41.8	5.7	13.4	16.6	6.2	2.7	30.8
7B	6.0	0.8	1.5	4.9	9.6	5.1	33.1	7.2	37.9	2.4	7.9	35.6	0.0
11B	5.6	0.6	3.7	4.8	7.3	4.9	37.7	5.3	10.2	21.2	6.2	10.9	29.4
19B	6.2	1.2	3.2	4.9	20.2	5.0	29.2	7.0	35.8	0.8	7.8	35.2	0.4

* The amounts of CaO used were:

250 mgm. for 5A, 7A, 11A, 18A
200 mgm. for 4A, 9A, 19A
100 mgm. for 5B, 7B, 11B, 19B

along with the ammonium salt, which will be just sufficient for the neutralization of the nitric and sulfuric acid formed, so as not to change the final reaction of the medium. Under these conditions we can study the influence of the sum total of the soil, including the physical, chemical and bacteriological conditions upon its nitrifying capacity. For the nitrification of organic matter, a smaller concentration of dried blood should be used, as suggested by Kelley (25). These methods have their advantages and disadvantages.

The advantages of the method of adjusting the initial reaction consist in testing the optimum potential nitrifying capacity of the soil, by bringing all the soils to the same reaction basis; thus the original and limiting acidity factors

TABLE 6

Influence of CaO and $CaCO_3$ on the nitrification of 50 mgm. of N as $(NH_4)_2SO_4$ in 100 gm. of soil after 28 days' incubation

PLOT NUMBER	SOIL ALONE		SOIL AND VARYING AMOUNTS OF $CaCO_3$									
			80 mgm.		160 mgm.		400 mgm.		800 mgm.		2 gm.	
	Initial reaction	NO_3-N	Initial reaction	NO_3-N	Initial reaction	NO_3-N	Initial reaction	NO_3-N	Initial reaction	NO_3-N	Initial reaction	NO_3-N
	pH	*mgm.*	*pH*	*mgm.*	*pH*	*mgm.*	*pH*	*mgm.*	*pH*	*mgm.*	*pH*	*mgm.*
5A	5.4	5.2	6.3	15.2	6.8	24.8	7.2	36.4	7.5	46.4	7.6	54.2
7A	4.8	0.05	...	....	...	....	...	....	...	.....	...	....
11A	4.4	0	...	....	6.0	7.6	6.9	5.6	7.6	2.4	...	....
19A	5.4	Tr.	6.4	1.2	7.0	5.9	7.5	1.8	7.7	0.5	7.8	0.6
5B	6.6	11.3	7.3	28.6	7.5	8.9	7.6	7.5	7.6	8.4	...	....
7B	6.4	4.4	...	....	...	....	...	....	...	.....	...	....
11B	6.2	2.8	6.5	8.8	7.2	19.5	7.5	15.4	7.7	16.8	7.8	15.9

PLOT NUMBER	SOIL AND VARYING AMOUNTS OF CaO									
	40 mgm.		80 mgm.		200 mgm.		400 mgm.		800 mgm.	
	Initial reaction	NO_3-N	Initial reaction	NO_3-N	Initial reaction	NO_3-N	Initial reaction	NO_3-N	Initial reaction	NO_3-N
	pH	*mgm*	*pH*	*mgm.*	*pH*	*mgm.*	*pH*	*mgm.*	*pH*	*mgm.*
5A	6.2	14.2	6.4	19.6	6.8	36.8	7.1	48.6	7.9	16.8
7A	...	....	6.0	1.2	6.8	Tr.	...	....	...	.....
11A	...	....	5.8	2.9	6.5	0.6	7.4	0	7.9	0
19A	6.5	2.6	6.6	8.5	6.8	1.4	7.6	0.42	8.2	0.12
5B	6.8	27.9	7.1	17.3	7.4	8.4	7.8	6.2	...	.....
7B	6.8	6.4	7.3	4.2	7.7	3.9	8.0	0.3	...	.....
11B	6.5	9.1	6.7	17.2	7.0	20.8	7.7	5.4	8.4	0.5

TABLE 7

Course of nitrate formation from 30 mgm. N as $(NH_4)_2SO_4$ and from 1 per cent of dried blood in 100 gm. of soil

PLOT NUMBER	NO_3-N FROM AMMONIUM SULFATE			NO_3-N AND NH_3-N FROM DRIED BLOOD								
				After 7 days			After 20 days			After 42 days		
	After 7 days	After 20 days	After 42 days	Reaction	NH_3-N	NO_3-N	Reaction	NH_3-N	NO_3-N	Reaction	NH_3-N	NO_3-N
	mgm.	*mgm.*	*mgm.*	*pH*	*mgm.*	*mgm.*	*pH*	*mgm.*	*mgm.*	*pH*	*mgm.*	*mgm.*
5A	1.7	4.2	8.6	6.8	50.7	8.9	5.6	57.9	43.5	5.2	47.6	71.0
7A	0.2	0.4	0.7	7.4	38.1	1.6	8.1	64.8	3.8	7.6	57.5	6.2
11A	0.3	0.3	0.3	6.9	44.1	3.6	7.8	81.6	4.9	7.8	86.2	5.0
5B	5.8	12.0	25.3	6.2	41.9	21.0	5.9	33.5	57.0	5.0	34.3	75.5
7B	1.5	7.2	13.6	7.6	45.2	4.9	7.5	44.7	5.9	7.3	41.0	5.5
11B	1.8	5.2	10.8	7.6	56.7	10.7	6.9	55.9	16.0	6.6	54.9	29.9

are eliminated, and a basis for comparison with neutral or faintly alkaline soils is obtained. Under these conditions we could use a test of the nitrifying capacity of the soil with both dried blood (0.1 or 0.25 per cent) and ammonium salts. The chief disadvantage of this method is that by adding lime to acid soils, sufficient to bring it to a pH of 7.0, we would have to use CaO, thus actually changing the physical and chemical condition of the soil as pointed out by Hutchinson and MacLennan (21), who found CaO to cause a partial sterilization of the soil, while $CaCO_3$ does not. It is also important to note that CaO causes a considerable increase in the hydroxyl-ion concentration in the soil, followed by a gradual decrease, as shown by Hoagland and Christie (20). $CaCO_3$ does not cause an appreciable change in reaction of neutral soils.

The chief advantage of the method of using a theoretical amount of $CaCO_3$ sufficient to neutralize all the acid formed from the nitrification of the $(NH_4)_2SO_4$ consists in the fact that, by this method, we can study the nitrifying capacity of the soil as such. At the same time there is enough basic material to prevent abundant acid formation from the oxidation of the ammonium salt so as not to produce in the more acid and poorly buffered (humus poor) soils a limiting acid reaction which will stop the activities of the nitrifying bacteria. However, as pointed out by Fischer (10), a theortical amount of $CaCO_3$ (200 mgm. for 132.2 mgm. ammonium sulfate) is not sufficient to neutralize completely all the acid formed, and we have to use 3.5 times the theoretical amount of $CaCO_3$. The disadvantage of this method consists in the fact that the oxidation of the ammonium salt and nitrate formation is slow and accumulative, while the $CaCO_3$ or CaO added in the beginning of the test, will produce an initial change in the reaction of medium, the carbonate less than the oxide, which will make conditions not strictly alike for the different soils. The use of a small concentration of dried blood (0.1 to 0.25 per cent) recommends itself also as of practical value, in view of the fact that the small amounts of ammonia formed will not be sufficient to change the reaction even of neutral or slightly alkaline soils to a point where free ammonia would be given off, unless in very alkaline soils. Both of these methods recommend themselves as more scientific than those which have been commonly used in the past.

Table 8 gives a summary of a typical experiment on nitrification in solution [medium no. 29 given by Fred (11)], in pure sand, moistened with the same solution, nitrification of the soil's own nitrogen, of small amounts of dried blood in the soil and of ammonium sulfate without and with CaO and $CaCO_3$.

The solution method shows definite differences, and it may, therefore, be used to great advantage. The use of pure sand, however, presents certain advantages over the solution method, particularly since more pronounced differences are obtained. On further study, this method has been modified as follows:

One hundred grams of sand, 210 mgm. $CaCO_3$ and 15 cc. of a medium containing 2.0 gm. K_2HPO_4, 1 gm. $MgSO_4 \cdot 7H_2O$, 0.4 gm. $FeSO_4 \cdot 7H_2O$ in 1000 cc. of water, are placed in 250-cc.

Erlenmeyer flasks, well mixed, plugged with cotton and sterilized, at 15 lbs. pressure for 1 hour. The ammonium sulfate is then added in solution, usually 3 or 5 cc. of water containing 30 mgm. of nitrogen. For inoculation 10-gm. portions of soil are used. The flasks are well shaken to obtain proper uniformity and incubated at 27–28°C. for 30 days. Either the solution or the sand method is used. The sand method has been found to give more uniform results.

TABLE 8

*A summary of results from methods of studying nitrification**

PLOT NUMBER	NITRATE NITROGEN DETERMINED															
	In solution with 10 gm. soil inoculum	In sand with 10 gm. soil inoculum†		In the soil itself	In soil + 0.1 per cent of dried blood		In soil + 0.25 per cent of dried blood		30 mgm. N as $(NH_4)_2SO_4$		30 mgm. N as $(NH_4)_2SO_4$ + 200 mgm. $CaCO_3$		30 mgm. N as $(NH_4)_2SO_4$ + 700 mgm. $CaCO_3$		30 mgm. N as $(NH_4)_2SO_4$ + CaO‡ with initial reaction adjusted to pH 7.0	
		200 mgm. $CaCO_3$	700 mgm. $CaCO_3$		After 5 days	After 28 days	Reaction		Reaction		Reaction		Reaction		Reaction	
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*pH*	*mgm.*	*pH*	*mgm.*	*pH*	*mgm.*	*pH*	*mgm.*	*pH*	*mgm.*
5A	0.76	7.80	10.70	2.50	1.64	10.40	5.1	24.40	4.8	5.20	4.6	24.40	6.9	34.80	6.9	28.20
6A	....	....	.....	1.98	1.60	9.60	4.9	25.20	4.8	4.30	4.5	31.20	7.0	33.60	6.7	28.00
6B	....	....	.....	1.14	3.00	9.40	5.3	25.30	4.9	11.60	5.9	30.90	6.8	32.60	6.0	23.30
7A	0.15	0.21	0.22	0.38	0.72	3.60	5.3	7.20	4.6	0.33	6.2	7.60	7.8	2.80	7.5	4.40
7B	0.38	1.44	1.04	0.60	1.20	10.80	5.0	18.30	4.8	6.20	6.2	16.95	7.3	20.60	5.1	17.70
11A	0.46	0.44	0.42	0.84	0.96	4.20	4.6	5.00	4.2	0.76	5.6	3.10	7.8	5.20	7.2	6.80
11B	0.84	5.70	13.50	0.94	1.52	10.60	4.8	21.20	4.8	3.80	5.2	26.10	7.3	27.50	6.05	22.00

* All cultures, except one-half of the group containing 0.1 per cent of dried blood, were incubated for 28–30 days at 28°C. Nitrate is given on the basis of milligrams of nitrogen as nitrate in 100 gm. of soil; pH is final.

† 100 gm. of pure washed quartz sand placed in 250-cc. Erlenmeyer flasks, moistened with 5 cc. of distilled water and sterilized for 2 hours at 15 lbs. pressure, with 200 or 700 mgm. $CaCO_3$. 10 cc. of the medium used for the solution studies containing 30 mgm. of nitrogen as $(NH_4)_2SO_4$ and sterilized separately, was added to each flask.

‡ The following amounts of CaO were used for 100 gm. of soil to bring the reaction of the soil to approximately pH 7.0–7.2:

Plot No.	*Mgm. CaO*	*Plot No.*	*Mgm. CaO*
5A	500	7B	50
6A	500	11A	375
6B	100	11B	250
7A	250		

Nitrification of the soil's own nitrogen gives important information. Nitrates are determined in the soil, soon after sampling. One hundred-gram portions of the soil are incubated in glass covered tumblers with optimum moisture (60 per cent of moisture-holding capacity) for 30 days. The nitrates are always determined in the moist soil, without previously drying it, due to the fact that the process of drying greatly increases the nitrate content of the soil, depending on the method of drying and type of soil.

Nitrification of ammonium sulfate in soil is best carried out in two ways: 1. The common method is to add 30 mgm. of nitrogen as ammonium sulfate to 100 gm. of soil and incubate with optimum moisture for 30 days. The nitrate formation under these conditions will give the maximum nitrate-holding capacity of the soil, or soil reaction and buffer content as influencing nitrate accumulation. 2. In addition to the ammonium sulfate 210 mgm. of calcium carbonate may be added to the soil. This allows us to study nitrification of ammonium salts under optimum conditions of reaction. In the case of alkali soils, this method may be superfluous.

It is also important to gain information on the nitrification of organic nitrogenous materials added to the soil. With an incubation period of 10 or 15 days at 27–28°C. 0.1 per cent of dried blood may be used. By using 0.25 per cent of dried blood, incubating 15 days, then determining the final reaction (pH value), ammonia content and nitrate, valuable information is gained.

By combining the information obtained from the study of nitrification by the five different methods, we can thus gain a proper picture of the process of nitrification in the soil and its bearing on soil fertility.

A detailed study of the correlation between results obtained from nitrification studies, numbers of microörganisms and crop productivity of the soil will be given in the following paper of this series.

SUMMARY

1. Nitrification of ammonium sulfate or other inorganic salts of ammonia in soils having different reactions cannot be used as a basis for comparison. This is due to the fact that the amount of nitrate accumulated in the soil, as commonly carried out under laboratory conditions, will depend on the initial reaction of the soil, buffer content and final reaction more so than on the bacteriological activities: less acid soils will allow a greater acumulation of nitrates than the more acid soils, under a given set of conditions.

2. In the oxidation of ammonium sulfate, nitric and sulfuric acid are formed. These acids increase the hydrogen-ion concentration of the soil, till a point is reached which becomes injurious to the activity of the nitrifying bacteria. In well buffered soils, such as those receiving large applications of organic matter, greater amounts of acid can be formed, before the injurious reaction is attained than in poorly buffered soils.

Nitrate accumulation from ammonium sulfate in the soil stops when the reaction has reached pH 4.4–4.8. The greater the buffer content of the soil the greater will be the amount of nitrate accumulated, even if the initial reaction is the same.

3. Nitrification of ammonium sulfate in the soil should be carried out, in laboratory studies, in the presence of sufficient basic material to neutralize the acids formed from the oxidation of the ammonium salt. $CaCO_3$ is to be

preferred to CaO, since the latter tends to change rapidly the reaction of the soil and, therefore, bring about various uncontrolled chemical, physical and biological changes.

4. The nitrification of dried blood, in concentrations commonly employed, namely one per cent, is not a good test for comparing different soils. This is due to the different reactions involved in the transformation of dried blood, particularly in alkaline and poorly buffered soils. The rapid decomposition of the dried blood brings about an abundant formation of ammonia, which is not sufficiently neutralized by acids or buffering agents in alkaline and poorly buffered soils. The free ammonia has an injurious action upon the activities of the nitrifying bacteria.

5. Either low grade nitrogenous materials should be used or a low concentration of high grade materials, for the study of nitrification of organic materials in the soil.

6. To get a thorough idea of the nitrifying capacity of the soil and have a basis for comparing the nitrification of different soils, no one single method is sufficient but a combination of the following methods is recommended, all of which should be used for each soil and each of which gives some information necessary to obtain a complete picture of nitrification in the soil.

a. Nitrification in solution, using 10 per cent of soil for inoculation, as recommended by Remy, Löhnis and others. The information obtained is valuable for a knowledge of the nitrifying flora of the soil, and the influence of the constitutents of the particular soil upon the nitrifying bacteria, when studied under standard laboratory conditions. The use of pure sand is to be preferred to the solution method: 100 gm. of pure washed sand + 210 mgm. $CaCO_3$ + 15 cc. of mineral solution placed in plugged flasks and sterilized in the autoclave. Three to five cubic centimeters of ammonium sulfate solution containing 30 mgm. of nitrogen and sterilized separately are added and each flask inoculated with 10 gm. of soil to be tested.

b. Nitrification of soil's own nitrogen. A definite amount of soil (100 gm.) kept in the laboratory for a definite length of time (30 days) at a definite temperature (25–28°C.), under optimum moisture conditions, will give us information on the forms of nitrogen present in the particular soil and the speed with which they are transformed into nitrates and thus made available for plant growth.

c. Nitrification of ammonium sulfate in the soil. By using a definite amount of nitrogen (30 mgm. in 100 gm. of soil), in the form of ammonium sulfate, and standard period of incubation, we get, from the amount of nitrate formed, an index on the buffering capacity of the soil in relation to nitrification. The final reaction should always be recorded.

d. Nitrification of ammonium sulfate in the presence of a theoretical amount of $CaCO_3$ [210 mgm. for 30 mgm. N as $(NH_4)_2SO_4$] necessary to neutralize all the acid formed from the complete oxidation of the ammonium sulfate into nitric and sulfuric acid. This gives an index of the nitrifying capacity of

the soil under optimum reaction conditions and forms an excellent basis for comparing nitrification with other soil bacteriological activities.

e. Nitrification of organic nitrogenous materials. If high grade materials are used (dried blood), 0.1-per-cent concentration should be used and a brief incubation period (10–15 days); 0.25-per-cent concentration and a period of incubation of 15 or 15 and 30 days gives important supplementary information.

By giving a definite weight to the information obtained by each of these five methods, a true picture of nitrification in soil may be obtained.

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AERATION METHOD FOR DETERMINING AMMONIA IN ALKALI SOILS

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In the investigation of bacterial activities in alkali soils at this station, it was imperative that a rapid method be found for the determination of ammonia. A satisfactory method for the determination must meet three requirements: First, all the ammonia in the soil must be removed and any added portion of ammonia must be fully recovered; second, organic nitrogenous material in the soil must not be broken down into compounds which yield ammonia; and third, the method must permit one to make several determinations within a reasonable length of time. Some of the methods which have been described prove impracticable because of complicated apparatus or the excessive time required for a complete determination. The copper flask distillation over magnesium oxide is quite extensively used, but cannot be applied to soils containing alkali salts because of the fact that the organic matter of the soil is attacked by the alkali and ammonia is split off. Potter and Snyder (5) have described a method which overcomes this difficulty. Their method calls for the use of a 25-gm. sample of soil and requires from 15 to 19 hours aeration at room temperature to complete a set of determinations. Davisson (1) and his associates have described an aeration method which is applicable to a larger sample and which requires but 2.5 hours for a determination. The objection to their method, however, is that an extract of the soil is used for the determination and not the soil itself, and the apparatus is somewhat complicated.

Matthews (4) describes an aeration method for the determination of ammonia in soils in which a 25-gm. sample is used and the aeration continued 6 hours at the rate of 300 liters per hour. He states that the time may be reduced to 3 hours in many cases. The apparatus is expensive and difficult to manipulate. Russell (6) tested several methods and applied heat to the aeration process.

Of the several methods reviewed, it seemed that the aeration method as described by Potter and Snyder was the most promising for the determination of ammonia in ammonification experiments with alkali soils if modifications could be made which would allow the complete determination of ammonia to be made on 100 gm. of soil in much less time than 15 to 19 hours.

A number of experiments were undertaken in which 100 gm. of soil were

used and in which an effort was made to hasten the removal of ammonia from the soil by increasing the temperature of the soil mixture during the aeration. In addition, experiments were carried out to show the effect of the increased temperature on the organic matter in the soil in the presence of definite amounts of alkali. The results are of especial interest to the biochemist.

METHOD

The aeration method for the determination of ammonia in urine was originally outlined by Folin (2), and the method applied to other solutions by Kober (3). Modifications were made by Potter and Snyder in their adaptation of the method to the determination of ammonia in soils. The apparatus has been described by them but a further description is briefly given. Figure 1 shows a cut of a unit of the aeration apparatus together with the trough used in heating the flasks during aeration.

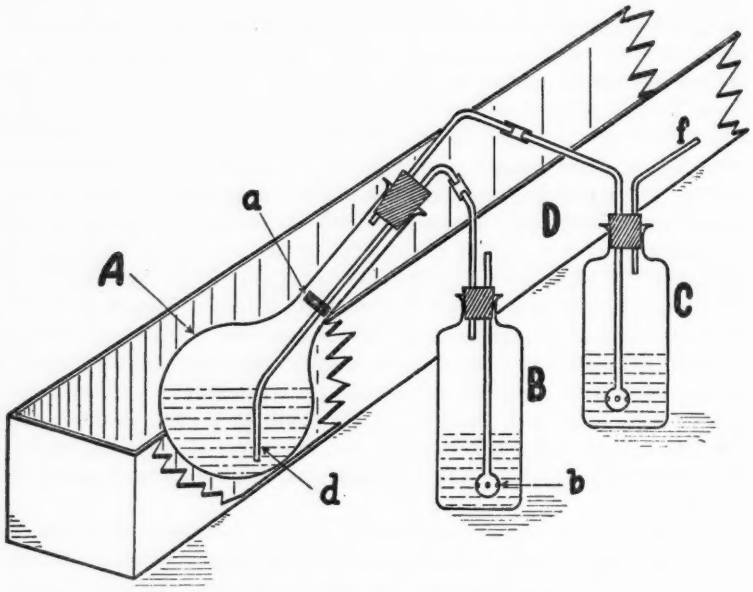

FIG. 1. AERATION APPARATUS

A. 800 cc. Kjeldahl flask
B. Dilute H_2SO_4
C. Standard H_2SO_4
D. Trough for hot water
a. Section of rubber stopper
b. Bulb for distributing air current
d. Air inlet tube
f. To another section or suction pump

The Kjeldahl flask (fig. 1) contains 100 gm. of the soil to be analyzed, approximately 4 gm. of sodium carbonate, 0.5 cc. paraffin oil, and approximately 300 cc. of ammonia-free water. When suction is applied at *f* a stream of washed air goes through the flask of soil suspension, thence through the flask of standard acid where the ammonia is caught. It may then be led through additional flasks of soil and standard acid, and thence into the suction pump. The section of rubber stopper *a* over the tube *d* prevents splashing into the neck of

the Kjeldahl flask. The trough *D* is filled with water which is maintained at a temperature of 75°C. It will be found that if the trough is six to eight feet in length a stream of hot water near the boiling point can be passed in at one end and out at the other and in this way maintain a temperature of 75 to 80°C. Since the aeration process has a tendency to constantly lower the temperature it is noted that the temperature at one end of the trough is higher than at the other end. Determinations at either end of the trough in this case will check provided the temperature at the cooler end is not below 75°C. In this work no differences were obtained between temperatures of 75 and 80°C., but temperatures below 75°C. gave irregular results.

To overcome the difficulty of maintaining a uniform temperature throughout the trough, the apparatus shown in figure 2 was designed, in which steam is used for heating the water. The rush of steam through the pipe *c* creates a suction which draws the water in at the side neck *b*, forces it through *c*, and thus back to *b* again. The pipe is placed in the bottom of the trough to one side and is not in the way of the Kjeldahl flasks. By this means the water may be held at the temperature desired. A union joint is placed in front of the steam valve which provides an easy means of disconnecting the apparatus when not in use.

The temperature of the water in the trough and the rate of air flow are the most essential factors in the rapid and complete removal of the ammonia from soils by this method. If the temperature is below 75°C. a longer time is required to remove the ammonia. The air flow must be regulated at such a rate that the soil in the flask is constantly held in suspension.

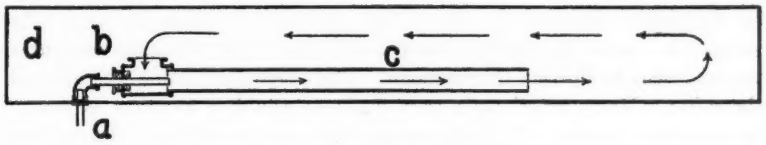

FIG. 2. HEATING ARRANGEMENT

a. Steam pipe
b. Side arm
c. One inch pipe
d. Trough

The end of the tube *d* (fig. 1) must be placed the proper distance from the bottom of the flask or the soil will settle regardless of the air flow. This distance was found to be about .25 inch. The section of rubber stopper assists in holding the tube in place and in addition prevents the contents of the flask from being mechanically carried into the neck. No estimate was made of the amount of air used in these determinations.

The suction was obtained by means of a Crowell vacuum pump driven by an electric motor. A condenser was placed between the last flask and the pump to remove the steam and thus prevent its condensing in the pump cylinder.

In setting up the apparatus it is well to arrange the Kjeldahl flasks in a slightly inclined position. The receiving bottles may then be arranged in a row by the side of the trough. The number of determinations that can be made at one time is limited only by the capacity of the vacuum pump. In this work six to ten determinations were made at one time, this being the maximum load the pump would pull and keep the soils well agitated.

The amount of acid chosen for the receiving flask must be in excess of the probable amount of ammonia present in the soil. Care must be exercised to have an excess in order to avoid complete nutralization, in which case ammonia will be carried into the next flask. In this work varying amounts of $\frac{1}{14}$ *N* acid were used and the volume in the receiving flask brought to approximately 125 cc. with distilled water. At the end of the aeration process the volume will be found to have increased by 50–75 cc. due to condensation. The contents were then carefully transferred to 500-cc. Erlenmeyer flasks and boiled on a hot-plate until the volume

decreased by approximately one-third in order to remove volatile compounds liberated from the soil which interfere with a sharp end-point in titrating. When cool the excess acid was determined by titration using methyl red as indicator.

Davisson and his associates state that the absorption of ammonia by this process is not complete. Our results, and likewise those of Potter and Snyder, indicate that all the ammonia is absorbed. Care should be taken in preparing the Folin tubes to make all the holes very small and of the same size. This gives small bubbles of air which are evenly distributed throughout the receiving flask. If the holes are not uniform some loss from splashing may result.

RESULTS

All experiments reported were made with a neutral silt loam soil of average fertility.

No attempt is made to present all the data obtained but only a few experiments to demonstrate the relative merits of the method. Many such experiments were made. The results obtained indicate clearly that the method may well be applied to soil biology. In the first series of experiments 5 gm. of magnesium oxide and 2 gm. of sodium carbonate were used to liberate the ammonia. The results obtained were in close accord with those obtained by the copper flask method and added quantities of ammonia were fully recovered. Later experiments demonstrated that 2 gm. of sodium carbonate was sufficient to liberate the ammonia. The magnesium oxide was omitted thereafter. Many experiments were made to establish the time necessary for complete removal of the ammonia. The results indicated clearly that 1 hour at 75°C. was sufficient. It is recommended, however, in routine analyses that the time be extended to 1.5 hours. It is quite probable that in the majority of determinations the ammonia was completely liberated within the first half hour. Amounts of sodium carbonate varying from 1 to 10 gm. were used, the results indicating that 2 gm. is sufficient to liberate the ammonia but that 10 gm. is not too great an excess.

Experiment 1

One-hundred-gram samples of soil received 43 mgm. nitrogen as ammonium sulfate and varying amounts of sodium carbonate. They were then aerated 1.5 hours. The results shown in table 1 indicate that 2 gm. of sodium carbonate liberated the ammonia from 100 gm. of soil when aerated 1.5 hours at 75°C. No difference is noted between 2 and 6 gm. of the carbonate, which indicates that no decomposition of the soil organic matter took place. This experiment was repeated aerating 1 hour. The results were in close accord with those shown in table 1 and indicate that an aeration of one hour is sufficient.

Experiment 2

Dried blood, glycine, alanine and asparagin were added to 100-gm. samples of the soil in definite amounts and the ammonia determined by aerating 1

hour. Dried blood, Witte's peptone, and asparagin were added to other samples along with 30 mgm. of nitrogen as ammonium sulfate. All samples received 10 gm. of sodium carbonate in the determination. Particular atten-

TABLE 1

Effect of sodium carbonate on the recovery of ammonia by aeration for 1.5 hours at 75°C.

NUMBER	SODIUM CARBONATE	NITROGEN AS NH_3*	AVERAGE
	gm.	*mgm.*	*mgm.*
1	1.0	38.8*	
2	1.0	41.2	
3	1.0	40.5	41.84
4	1.0	43.6	
5	1.0	45.1	
6	2.0	42.5	
7	2.0	44.1	
8	2.0	43.8	
9	2.0	43.7	43.65
10	2.0	44.2	
11	2.0	43.6	
12	3.0	44.4	
13	3.0	43.7	
14	3.0	44.1	43.88
15	3.0	42.8	
16	3.0	44.4	
17	3.0	43.9	
18	4.0	44.3	
19	4.0	43.8	
20	4.0	43.4	43.48
21	4.0	43.7	
22	4.0	42.2	
23	5.0	43.8	
24	5.0	44.6	
25	5.0	44.8	43.92
26	5.0	43.0	
27	5.0	43.4	
28	6.0	43.7	
29	6.0	44.4	43.85
30	6.0	44.0	
31	6.0	43.3	

* Each sample received 43 mgm. of nitrogen as ammonium sulfate.

tion was paid to the temperature of the water, keeping it around 85°C. The results shown in table 2 indicate that none of the compounds under test were decomposed to yield ammonia by the aeration process. The sample of soil containing the peptone yielded 3.7 mgm. more nitrogen than was added as

ammonium sulfate. The peptone was found to contain 2.5 mgm. nitrogen as ammonia when determined by distillation over magnesium oxide. These results indicate that soil organic matter is not decomposed by this method to yield ammonia in ammonification experiments.

TABLE 2

Effect of aeration for 1 hour at 85°C. on organic compounds

NUMBER	TREATMENT OF SOIL	NITROGEN AS NH_3	AVERAGE
		mgm.	*mgm.*
1	10 gm. Na_2CO_3 1 gm. dried blood	0.6	0.7
2		0.7	
3		0.5	
4		0.9	
5	10 gm. Na_2CO_3 1 gm. dried blood and 30 mgm. N. as $(NH_4)_2SO_4$	30.7	31.1
6		30.5	
7		31.5	
8		31.0	
9		31.3	
10		31.6	
11	10 gm. Na_2CO_3 2.5 gm. Wittes peptone and 30 mgm. N. as $(NH_4)_2SO_4$	34.4	33.7
12		32.9	
13		33.8	
14	10 gm. Na_2CO_3 14 mgm. N. as Asparagin and 30 mgm. N. as $(NH_4)_2SO_4$	30.8	31.3
15		31.7	
16		31.3	
17	10 gm. Na_2CO_3 0.1 gm. glycine	1.4	1.45
18		1.5	
19	10 gm. Na_2CO_3 0.12 gm. alanine	1.1	1.0
20		0.9	
21	10 gm. Na_2CO_3 0.188 gm. asparagin	1.3	1.4
22		1.5	

Experiment 3

One-hundred-gram samples of soil received 2 gm. of finely ground dried blood and 26 gm. of water and were then incubated 1 week at 28°C. At the end of this period ammonia was determined by the method above described, aeration being continued 1 hour. The results shown in table 3 indicate that the yields of ammonia from the samples agree very closely. The average yield of ammonia when 2 gm. of sodium carbonate are used in the determinations is greater than the average where 4 or 6 gm. are used. No significance can be attached to their slight variation since the ammonia content of the

samples varies slightly. A variation of 1–2 mgm. of nitrogen as ammonia is negligible in ammonification experiments where an amount as high as 120 mgm. is involved. The results clearly indicate that only the ammonia in the soil is liberated by the aeration process and that no decomposition of the organic matter to yield ammonia is taking place. These data also indicate that 2–6 gm. of sodium carbonate may be used in the determination. This is a matter of considerable importance since it eliminates weighing the carbonate in routine determinations.

TABLE 3

Effect of varying amounts of sodium carbonate on ammonia content with aeration for 1 hour at 75°C.

NUMBER	SODIUM CARBONATE	NITROGEN AS NH_3	AVERAGE
	gram	*mgm.*	*mgm.*
1	2.0	121.9	
2	2.0	117.9	
3	2.0	120.7	
4	2.0	124.7	121.5
5	2.0	123.6	
6	2.0	120.4	
7	4.0	119.0	
8	4.0	117.0	
9	4.0	122.5	
10	4.0	124.2	121.0
11	4.0	121.6	
12	4.0	122.0	
13	6.0	121.4	
14	6.0	119.8	
15	6.0	123.0	
16	6.0	119.4	120.4
17	6.0	119.9	
18	6.0	119.2	

SUMMARY

1. Complete aeration of the ammonia from 100 gm. of soil was obtained in 1–1.5 hours at a temperature or 75 to 85°C.

2. No ammonia is formed from the organic matter in soil in the presence of alkali concentrations and temperatures used in these experiments.

3. The aeration method can be used for the determination of ammonia in ammonification experiments with alkali soils.

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METHOD FOR ESTIMATING ADSORBED BASES IN SOILS AND THE IMPORTANCE OF THESE BASES IN SOIL ECONOMY[1]

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A part of the bases (calcium, magnesium, potassium, sodium, and ammonium) which may occur in the clay-humus complex of the soil is present in a replaceable or exchangeable form. It is possible to demonstrate this fact by treatment of the soil with a solution of a salt of one of these bases (for example with ammonium chloride). We then find a replacement of calcium, magnesium, potassium, or sodium of the soil by equivalent proportions of the ammonium from the solution. The replacement process is reversible; ammonium ions from the solution are capable of replacing Ca, Mg, K, and Na ions from the soil; but these in turn can also replace the soil ammonium to a certain extent. As soon as equilibrium is established we find in both the soil and the solution NH_4, Ca, Mg, K, and Na ions. Solutions of $NaNO_3$, $CaSO_4$, etc, behave in a similar manner.

Experiments here reported show that equilibrium is very quickly attained in this "replacing" process. This fact indicates that here we are dealing with a reaction between the solution and the easily accessible or surface particles of the clay-humus complex. If the bases on the interior of the adsorbing particles were concerned either partly or wholly in this reaction equilibrium would only be reached slowly, because diffusion occurs very slowly in solid bodies. Other phenomena would also make it appear that the particles which take part in this process are present in the ionic form. We may, therefore, consider that at the boundary surface between the soil as the solid phase and of the soil solution as the liquid phase, replaceable cations occur,—that is, replaceable cations are adsorptively bound to the adsorbing soil particles. The idea then occurs that the exchangeable cations and the adsorptively-bound cations may be identical.

Explanations of this base-adsorption are to be sought in chemical relations: chemical compounds are formed between the adsorbed bases (Ca, Mg, K, Na, and NH_4) and the clay- and humic-acids of the soil. The fact that only the molecules in the surface layer react to form these chemical combinations characterizes the reaction as an adsorption process.

[1] For original paper, tables and references, see Verslagen van Landbouwkundige Onderzoekingen der Rijkslandbouwproefstations, 1920, no. 24, p. 144–250.

Since the chemical combination only occurs at the surface of the adsorbing clay-humus particles we can well understand that, from calculations based on the mass of the whole particle, no combination occurs in simple stoichiometric proportions as is the case in ordinary chemical reactions of this kind. This difference in extent of reaction between adsorption-combinations and others of a purely chemical nature gradually becomes less as the adsorbing particles become smaller; that is, as the "specific" surface of the particles becomes greater. If the particles are so small as to approach the dimensions of a molecule the adsorption process than becomes a chemical reaction. This apparently is the case with permutite. According to Schulze, at least, every molecule in permutite particles resides on the surface of the particle. Permutites are, therefore, adsorption combinations in stoichiometric proportions. This explanation clears up the controversy between Gans, Stremme, and others. In soils, such an evolution from adsorption to chemical combination is quite possible, especially with certain of the humus-substances. Ordinarily the particles are no smaller than colloids; most of them are larger even than 0.1μ as investigation has shown. The humus constituents occur in all degrees of fineness, and some of the particles are no larger than molecules.

The adsorbed bases play important parts in the processes taking place in the soil. It is therefore desirable to ascertain the amounts of these bases present in soils. The ordinary methods of soil examination are inadequate for the purpose.

It is apparent that any method for estimating the adsorbed bases depends on the properties of these bases towards other bases in the solution. Since the "exchanging" process is reversible the method chosen must be based upon a leaching process. Even on digestion with a moderately strong solution of ammonium chloride (Meijer method) all the adsorbed calcium does not go into solution. Now the question arises whether by leaching soils with solutions, for example, of ammonium, potassium, or sodium chloride, only the bases from the surface of the soil particles are removed or whether they are also removed from the interior of the particles in appreciable amounts. If this were the case then the exchange process in the soil would occur in this way: at first a fairly large amount of the base elements would go into solution, while with further leaching smaller quantities go into the solution. If only the adsorbed elements are dissolved the exchange process would, for all practical purposes, soon be complete. In order to study these questions, an exact investigation was conducted with clay soils and with sandy loams, all free from calcium carbonate. The experiment showed that a point was reached very quickly at which no more bases were given up to the solution. On leaching the soil with ammonium chloride, potassium chloride, or sodium chloride, therefore, only adsorbed bases go into solution.

As further ground for this conclusion, the speed of the exchange process was investigated. If only the adsorbed bases are replaceable equilibrium should be quickly reached. For the present only the exchangeability of calcium in a clay soil and in two sandy loams will be considered. For esti-

mating this capacity for exchange of bases, a weighed quantity of soil was treated with a definite amount of the chloride solution by shaking. Determinations were than made of the amount of calcium present in the solution after periods of 5 seconds, 1 minute, 1 hour, 1 day, 1 week. With the clay soil equilibrium was reached in less than 1 minute. With a sandy loam soil only 0.01 per cent calcium went into solution after 5 minutes. From consideration of these results it was seen that of the total quantity of calcium which could go into solution under these considerations in the clay soil, 97 per cent went into solution in the first 5 seconds, and for the sandy loams 90 per cent was in solution at the end of 5 minutes. Such results could only be obtained where the exchange process is confined to the easily accessible adsorbed bases.

The solubility of $CaCO_3$ in water is low. In presence of NaCl and KCl it is not appreciably increased, but is increased to a considerable extent by NH_4Cl solution. On leaching soils containing calcium carbonate, with solutions of NH_4Cl, KCl, and NaCl, more or less of the soil carbonate of lime, in addition to adsorbed calcium, is dissolved.

It was shown in an extensive investigation that the quantity of calcium carbonate which goes into solution on leaching the soil with NH_4Cl solution decreases as leaching proceeds. In the first liter of extract, therefore, more of the $CaCO_3$ is dissolved than in the second. When using solutions of KCl or NaCl only small amounts of the carbonate dissolved. It was observed that with these two solutions this quantity is practically proportional to the quantity of the solution used for leaching (up to two liters). In the second liter there is practically as much $CaCO_3$ dissolved as in the first. On leaching with KCl or NaCl solution the difference in the calcium content of the first and of the second liter represents the amount of replaced calcium from the soil.

It was found that in the soils examined no $MgCO_3$ was present.

METHOD FOR ESTIMATING ADSORBED BASES

1. Estimation of adsorbed lime and magnesia

Twenty-five grams of soil (for mixed sandy loam soils 50 gm. may be used) were shaken in a beaker with 100 cc. of a warm normal solution of NaCl. This was occasionally shaken and allowed to stand over night. In this way the sample was thoroughly saturated with NaCl solution. The liquid was then poured through a filter into a liter flask, the mass of soil brought quantitatively on the filter and treated with successive portions of the solution. The filter was allowed to empty between each addition of NaCl solution. If the first portion of the filtrate was cloudy it was run through the filter again. When the flask was filled to the mark the funnel was placed in another liter flask and treatment continued until the second flask was also filled to the mark. The calcium content of the two filtrates was then determined. The difference in the calcium content of the first- and the second-liter portions corresponds to the replaced calcium. Adsorbed magnesium may be estimated in the same way although this is more conveniently determined as under (2).

The second liter contains only traces of magnesium; and in soils which do not contain calcium carbonate the second liter is practically free of calcium.

2. Estimation of adsorbed magnesium, potassium, and sodium

These elements were determined in a manner similar to (1) except that 25-gm. portions of the soil were leached out with successive portions of normal ammonium chloride and the washings were collected in two half-liter flasks.

DISCUSSION

In this method the adsorbed bases are represented by the difference in quantities removed in the first and the second liter or half-liter portions. Even if further study should show that besides the adsorbed bases small quantities of that class of bases which I have designated as "acid-soluble" are also removed, the method would still permit the satisfactory determination of the adsorbed bases. The small quantities of acid-soluble bases removed would be proportional to the amount of leaching liquid. Thus if sodium chloride removed small quantities of acid-soluble calcium and magnesium the amounts removed in the first- and second-liter portions would be equal. Therefore the difference in calcium or magnesium content of the first and second liters would represent the exchangeable calcium or magnesium. The same considerations apply to ammonium chloride except that we deal with half-liter portions. From the same line of reasoning we need expect no serious difficulty due to impurities in the sodium chloride or ammonium chloride.

The adsorbed calcium was estimated in a large number of clay soils as described above, while for a part of these soils the adsorbed magnesium, potassium, and sodium was also estimated. The results are stated as the content of exchangeable bases, in percentages. From these percentages are calculated the content of exchangeable bases expressed in milligram-equivalents per 100 gm. of air-dry soil, and per hundred adsorbed ions. Thus, an average of the soils examined contained per 100 gm. of soil: 30.0 m.e. Ca, 5.0 m.e. Mg, 0.8 m.e. K, and 2.5 m.e. Na,—a total of 38.3 milligram-equivalents in 100 gm. of soil. Then in one hundred adsorbed cations there are 79 Ca, 13 Mg, 2 K, and 6 Na ions. The divalent ions predominate, calcium being the most prominent one.

Two sandy loam soils were examined for adsorbed bases. In the humus, there were 76.3 Ca ions, 13.1 Mg ions, 3.0 K ions, and 7.6 Na ions for each hundred adsorbed cations. In these soils the divalent ions also predominate.

The term "acid-soluble bases" designates the portion of Ca, Mg, K, and Na which goes into solution when the soil is treated with strong HCl, after deducting the adsorbed bases and the water-soluble chlorides, carbonates, etc. All clay soils examined have a low acid-soluble Ca and Na content (averaging 0.251 and 0.270 per cent) as compared with a high acid-soluble K and Mg content (1.340 and 0.826 per cent). Of the total calcium (adsorbed

and acid-soluble), 76.9 per cent was in the adsorbed condition. Of the total Mg, K, and Na contents only 5.6, 2.6, and 10.9 per cent respectively, were adsorbed. These bases occur for the greater part in the acid-soluble form, as contrasted with calcium, which is for the greater part adsorbed.

Two sandy loams were examined for acid-soluble bases. These differed from the clay soils in that the calcium ranked highest as the acid-soluble base. In the clay the greater part of the bases was in the acid-soluble form with the exception of calcium. With the sandy loams, the opposite relations were observed. For a fair comparison the *sum* of the total bases, should be compared with the sum of the adsorbed bases (both expressed in equivalents). Thus 100 gm. of a clay soil contained 137.7 m.e. of total bases and only 35.4 m.e. of adsorbed bases. Of the total bases (soluble in strong HCl) present in the clay soil about 25 per cent occurred as adsorbed bases. The sandy loam soils contained 36.9 m.e. of total bases, and 21.8 m.e. (or 59 per cent) of adsorbed bases. In the humus the major part of the bases occurred in the adsorbed form, while in the weathered mineral-complex (clay) the greater part occurred in the acid-soluble form. It is evident that the reason for this distinction is to be found in the variation in size of the particles in the mineral and in the organic weathered-complex. The smaller the particles the greater the ratio of surface to mass and naturally the greater the amount of adsorbed substances present.

The great importance of adsorbed bases in the soil processes is recognized both from the standpoint of clay soils and of sandy loams.

The amounts of adsorbed bases in milligram-equivalents designated as S varied in the clay soils examined from 23.3 to 48.9 (with the exception of a very low value of 12.4 for a soil B38) and with the loam soils examined, from 8.4 to 21.8. These values depend primarily on the content of adsorbing substance in the soil (clay-humus), and further, they decrease in the course of years due to action of plants and the percolating action of rain water.

In the moist climate of Holland the soils are adsorptively unsaturated.

The loss of a part of the adsorbed bases is partly caused by physiological agencies and partly by colloidal-chemical means. If a sufficient amount of adsorbed bases is lacking, the soil is in such a condition of unsaturation that the adsorbing soil complex can not efficiently function as the pH regulator. The soils then become acid. Besides, a change in the colloidal equilibrium of the soil occurs following such a desaturation of the soil, and this causes considerable modification of its physical nature (formation of hard-pans). With clay soils the effects of this desaturation on the colloidal-chemical processes are more noticeable. With loam soils the physiological effects are noticed to a greater or lesser extent. With these there is as much probability of a strongly alkaline as a strongly acid reaction. in the soil solution. The range of optimum acidity of the soil solution, the acidity being expressed by the value pH, is more quickly changed in either direction with soils high in humus than is the case with clay soils. This difference may be due to the difference in strength of the clay- and the humic-acids.

The value of S (sum of adsorbed bases expressed as milligram-equivalents per 100 gm. of soil) gives us no positive insight into the real character of the soil. To fully understand this a knowledge of the degree of saturation of the soil is necessary. This term is used by the author to designate the ratio of the actual quantity of adsorbed bases in the soil S to the possible degree to which this might be extended; that is, to the amount of bases which the soil is capable of adsorbing T. A satisfactory procedure for the determination of T has not yet been worked out. A relation evidently exists between the value T and the clay-humus content, or, T varies with the content of clay humus substance. The values calculated on this assumption, which do not give the actual saturation capacities but are only proportional to them, were determined for a series of clays and for one sandy loam soil. With clay soils, the degree of saturation varied from 67 with the younger peat soils to 20 with older soils and "Katteklei"; in sandy loams it ran from 193 with calcareous valley soil to 64 with an old valley soil.

The colloidal chemical equilibrium depends not only upon the degree of saturation of the soil but also on the relation of the adsorbed bases one to another. For practical purposes, to these various relations of adsorbed cations may be ascribed that well known agricultural fact that calcic fertilizer materials act favorably on soil structure while sodium compounds have the opposite effect, and make the soil "sticky." Theoretically, the difference in effect of lime fertilization on the soil structure on the one hand, and the effect of fertilization with sodium compounds, on the other, is due to formation of gels in the first case and sols in the latter. Then the great variation in the deflocculating power of the di- and mono-valent cations on clay suspensions and humus fluids is due to the colloidal chemical effects of the adsorbed monovalent and divalent cations in the soil. Changes in these relations lead to changes in the equilibrium and influence changes in the soil structure.

The Rothamsted experiments have shown the unfortunate results of continued yearly applications of nitrate of soda on the soil structure. Likewise the ill effects of flooding fields with sea water is well known. Examination of such soils shows that they differ markedly from normal soils. As a rule they contain per 100 adsorbed cations only 56.9 Ca ions and 20.2 Mg. ions, or 77 divalent cations, against 23 monovalent cations. In normal soils the figures are 92 divalent to 8 monovalent cations.

Salts in sea water not only affect the adsorbing substances of mineral nature but also those of organic or humus nature. The effects are mainly on the calcium (gels) and sodium (sols) humates. The presence of the humus sols in soils treated with sea water is shown by extraction of such soils with water. Normal calcium-clay soils give bright yellow colored extracts; the extracts from the sodium-clay soils are yellow to brown in color.

It is evident that variations occur in the relations of adsorbed cations of water-borne soils according to whether the soil was deposited by fresh or salt water. In the latter instance, such as the so-called "Kwelder" soils, the soils

are distinguished by their high content of adsorbed monovalent cations (50 divalent and 50 monovalent, generally) while the fresh water action on soils leaves them with much smaller amounts of the monovalent cations. As the content of exchangeable calcium increases, the content of exchangeable sodium and of calcium carbonate decreases. One experiment seems to show that salt water clays always contain considerable exchangeable sodium. In this way soils which have been in contact with fresh water may be distinguished from those which have been in contact with salt water.

At the present it is generally assumed that plant nutrients are distributed through the soil in a slightly water-soluble form, so that neither a complete removal by leaching on the one hand or too strong a concentration of the soil solution on the other, can occur. But water, and especially carbonated water can always take up small quantities. As plants withdraw the dissolved materials from this dilute solution they are replaced to a greater or lesser extent by weathering of silicates or humus materials. It is worthy of note that this explanation of withdrawing of nutrients from the soil by the chemical changes taking place between adsorbing material of the soil and the substances dissolved in the soil water has not been elaborated before. The investigations of Prianischnikow have directed attention to these changes. From his investigations it was shown that potassium absorbed in potash-permutite could not be utilized by plants without the action of the other salts of the soil solution. The author has shown, moreover, that adsorbed potash is just as available for the plants' needs as the water-soluble potash. The same conclusion was also drawn as regards the ammonia of ammonium permutite. It does not matter if *only* the adsorbed bases are assimilated. Kellner has made a study of this and concludes that potash and calcium in the dissolved or absorbed condition can serve as nutrients for legumes but cannot be taken up from difficultly-soluble compounds by the roots.

From evidence presented we cannot say that it is principally the adsorded substances which are adsorbed. Perhaps the opinion of Adolph Meyer may well be considered, i.e., that the substances in the adsorbed condition are *not* the only ones available. But it seems that the adsorbed bases are of fundamental importance in absorption through the roots of plants. From this standpoint the determination of these bases deserves thorough attention.

In this connection it is well to give attention to the experiment and observations of Ehrenberg who has studied the "calcium-potassium-ratio." This ratio concerns to a great extent the great differences in content of adsorbed calcium and potassium in clay soils, and considers the general relation of the adsorbed bases with one another. "Kwelder" soils which are high in sodium and poor in calcium are spoken of as of the "calcium-sodium-type."

The question arises as to whether there is a reciprocal transfer between the adsorbed and acid-soluble bases. This cannot possibly occur to any great extent. If it were the case then we could not explain why the content of adsorbed and of acid-soluble bases in a soil had not become equalized after

existing so closely together for these hundreds of years. There should, for example, not be such great difference between the adsorbed magnesium (0.08 per cent) and potassium (0.024 per cent) and the acid-soluble magnesium (1.34 per cent) and potassium (0.826 per cent). Then also the lowering of the degree of saturation of soil, which actually occurs and to a considerale extent, could not be explained so long as these soils have such an excess of acid-soluble bases. From these considerations we must consider two forms of bases, the exchangeable or adsorbed bases, and the acid-soluble bases. Between these two classes no appreciable exchange occurs.

The influence of adsorbed bases on the soil processes, as previously mentioned is remarkable. The small amount of adsorbed potassium found in the soils examined, and averaging only 0.024 per cent is of greater importance for plant nutrition than the 0.826 per cent acid-soluble potassium. The degree of saturation of soils depends on the content of adsorbed bases. While this content in the course of years might decline only a little in comparison with the total-base content, yet even a small decrease has a great effect on the processes, physiological as well as colloidal-chemical, which take place in the soil. There is further the relation between the monovalent and the divalent cations which is also involved in governing the colloid-chemical equilibrium in the soil. In normal soils the adsorbed monovalent cations are outnumbered by the total number of adsorbed cations. The proportion of acid-soluble divalent cations to monovalent acid-soluble cations is not of any significance.

Even if it should be shown by future investigations that in my discussion the importance of adsorbed or exchangeable bases has been given too much emphasis and the acid-soluble bases undervalued, enough has been shown to indicate that the former are of greater importance to soil properties than the latter. At any rate the estimation of exchangeable or adsorbed bases should have a place in any extensive scheme of soil examination.

NODULE-PRODUCTION KINSHIP BETWEEN THE SOY BEAN AND THE COWPEA

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INTRODUCTION

There has been much speculation as to the relationship of the organisms from the nodules of the numerous species of legumes. Moore (13) has presented the extreme possibility of relationship between legume bacteria, namely, that only slight physiological differences exist between strains due to association with a certain legume species, which differences can be destroyed by cultivation upon artificially prepared media. This conclusion has not been borne out by later experimental work with improved methods.

HISTORICAL

The data to be reported in this paper bear only on the varieties or strains of *B. radicicola* Bey. isolated from soy bean *Soja max*, Piper, and cowpea *Vigna sinensis* (L.) Endl., and species related to the cowpea on account of the reported interchangeability of their nodule-forming organisms, for which reason the literature pertaining to groups other than these two will not be taken into consideration except when it has a close bearing on the subject.

There is a preponderance of evidence obtained by field observations and laboratory investigations to the effect that the bacteria which produce nodules on the soy bean will not produce them on the roots of any other common legume.

Kirchner (7) in observations made at Hohenheim, Germany, during a number of years failed to find nodules on the soy bean. Hiltner and Stormer (4), Stutzer (19) and Simon (18) confirm this condition. On the other hand, Kirchner (7, footnote) refers to Cohn's work at Breslau when he found nodules on the roots of plants of soy beans growing in soil to which no inoculating material of any kind had ever been applied.

As a result of pure culture studies Simon (18) concludes that *B. radicicola* from soy bean does not cross inoculate with lupines and other legumes but his series of plants does not include the members of the cowpea group. This lack of relationship is in agreement with his serological work and that of Krüger (8).

The absence of the cowpea nodule-organism from certain soils in Austria is reported by Gross (3) and Simon (18) reports for German soil on which he experimented, the absence of nodule formation of *Lespedeza striata*, (Thunb.) H. and A., *Vigna sinensis*, and *Dolichos* species, all of which belong to the cowpea group.

In the United States, it has been observed over a period of years at experiment stations and on farms that it is usually necessary to introduce soy bean bacteria when soy beans are being planted for the first time. Otis (14) found that the soy bean organism was lacking in the soils at the Manhattan station yet Hitchcock (5) reports at about the same time, the presence of *Meibomia sp.*, *Lespedeza sp.*, and nodule-producing *Cassia sp.* growing in the vicinity of the station. Lewis and Nicholson (9) found that the cowpea organisms were abundant in the soils of the experiment station farm whereas soy bean plants did not have nodules on their roots. Lipman (10) reports that at Hammonton, N. J., without the introduction of inoculation soy beans produced no nodules whatever although cowpeas had numerous nodules on their roots. Hopkins (6) makes a similar report. Perhaps the literature of other experiment stations might tend to make the case more complete but sufficient of the field observations have been given to show the unanimity of the results.

These field observations may be supplemented by the pure-culture work of Garman and Didlake (2) from which they conclude that the soy bean nodule bacteria are a distinct species, not forming nodules on cowpea, garden bean or garden pea. Burrill and Hansen (1) reach practically the same point of view as the result of vegetative tests but find between the cowpea and soy bean organisms certain similar characteristics such as rate of growth, color and consistency.

Morphological studies by Löhnis and Hansen (12) and by Shunk independently (17) have shown that the soy bean and cowpea nodule bacteria possess polar flagella in contradistinction to the peritrichous flagella of cultures of *B. radicicola* from other legumes shown to be unrelated to these two by vegetative test. Another similarity has been brought out by Löhnis and Hansen (12) namely, the effect of the peritrichic and monotrichic bacteria on sterile milk. The former cause a clear zone to form on the top of the milk in tubes but the latter in a reasonable length of time do not apparently alter the milk.

METHODS

Isolation of cultures

In view of the extreme variance of the data to be submitted with that already on record it has been deemed advisable to give somewhat in detail the methods employed in securing these results although many of the processes are common and quite well known.

Nodules selected for inoculum were washed, sterilized for about five minutes in mercuric chloride solution made up at the rate of 1 gm. of chloride, 500 cc. water, and 2.5 cc. HCl. The nodules were then washed with five different lots of sterile distilled water to eliminate the mercury salt. The above method was used only on nodules from which cultures were isolated previous to five years ago, subsequent isolations were made from nodules which were first thoroughly washed in tap water and then five times in sterile distilled water. Sterile conditions were maintained throughout the isolation by flaming the nodules using a sterilized scalpel to cut open the nodule for removing a small amount of inoculum from its interior. This inoculum was placed in sterile distilled water and shaken thoroughly following which it was further diluted, and transferred to sterile Petri dishes by sterile pipettes. Previous to five years ago Moore's snythetic nitrogen-free agar (13) was used in this work. Since then, however, and at the present time Löhnis soil extract mannite agar (11) has been employed. Plates were incubated in an insulated chamber

usually at living room temperatures ranging from 20° to 27°C. Characteristic colonies were selected and transferred to agar slants. The number of times a culture has been plated is dependent on the use to which it has been put. Some of the older cultures used in this work have been plated as high as twenty times but the usual number of platings has been but two or three; usually after the second plating the stock was multiplied in agar and broth for carrying on vegetative tests.

Vegetative tests

Building sand was washed in running water for one day, drained and placed on a clean table to air-dry. After drying in air the sand was put into a drying oven for a day or until thoroughly dry after which it was sieved to secure a uniform product. This sand was mixed with a small percentage of calcium carbonate and put in 32-ounce wide-mouth flint-glass bottles, 412 gm. per bottle. The sand was then moistened with 100 cc. of a nutrient solution made from a formula modified from Sachs (16) as follows:

Tap water	10,000 cc.
Calcium monophosphate	0.5 gm.
Sodium chloride	2.5 gm.
Potassium chloride	2.5 gm.
Magnesium sulfate	5.0 gm.

These bottles were closed with absorbent cotton plugs and sterilized for one hour at 17 pounds steam pressure.

It is practically impossible to obtain seed, especially the larger sized seed, which are free from foreign organisms but it is not a difficult matter to sterilize such seed to eliminate the legume organism. As a matter of fact, I have found that very few commercial leguminous seed carry any legume bacteria on or in their seed coats. The process of sterilization which was used in this work included both the hydrogen peroxide method of Robinson (15) and the mercuric chloride method previously mentioned in connection with the sterilization of nodules. The time of exposure with the latter disinfectant was doubled when seed were sterilized.

Seeds which had been sterilized were introduced into the sterile sand in bottles by a sterile platinum spoon in the protection of a culture room. These seeds were covered by agitating the sand with blows of the hands on the outside of the bottle. When growth was first apparent the culture to be tested was added in the culture room by means of a sterile pipette after which the bottles were transferred to the greenhouse in order to give the inclosed plants the light necessary for best growth. The cotton plugs were covered with paper to prevent evaporation and keep dust and other foreign substances from settling on them. Since the plants only grew a few months, it was unnecessary to add water. Water transpired by the plant was condensed when it came in contact with the shoulder of the bottle and thus was carried back to the sand to be used again by the roots of the plant. It is recognized that the plants in such bottles

as these are under very abnormal conditions, expecially as regards the process of photosynthesis and respiration but there occurred sufficient growth of root and shoot in the short period of experiment to allow for the development of nodules. Each series of bottles was checked by controls which were untreated with the legume organisms and in no case have nodules been found on the roots of plants from these bottles. This, it is assumed, is sufficient evidence to warrant the statement that the seed planted were sterile with reference to *B. radicicola*. Each test was at least made in quintiplicate.

OBSERVATIONS

In an endeavor to group the nodule organisms of Adzuki bean, *Phaseolus angularis* Willd. in 1917 under pure culture conditions it was observed that nodules were produced on Adzuki bean roots by cultures of *B. radicicola* from soy bean, cowpea and Japan clover, and that a culture of nodule bacteria from Adzuki bean not only caused nodules to form on cowpea but also on soy bean. Other experimental work demonstrated similar relations between certain cowpea and soy bean cultures which led to the belief that there might be an unrecognized kinship between these organsms.

Further experimentation gave results which strengthened the case very much. The facts as gathered are presented in table 1.

DISCUSSION

The nodules formed by soy bean cultures on cowpea roots have been observed to be as normal in appearance and structure as those produced under similar conditions on the natural host. On the other hand, in a few instances nodules on soy bean roots produced by cowpea bacteria were more disk shaped than the normal nodules which were spheroidal in shape.

The facts presented in the foregoing table indicate a physiological relationship between the root nodule organisms from cowpea and related species and those of the soy bean. It will be noted that in every instance the soy bean organisms produced nodules on a variety of the normal host species as well as on the roots of a variety of cowpea. Cowpea bacteria, however, have given rather inconsistent results, characterized by numerous failures to produce nodules on the roots of soy bean plants.

The question which arises first is one which concerns the purity of the cultures. In view of the numerous purifications by plating of most of the soy bean cultures without altering their ability to function on the roots of both legumes, and the consistency with which the cross takes place with cultures of varying ages and from various sources, it seem almost an absurdity to consider that the results may be due to mixed cultures. If they are mixed they were probably in this condition in the nodule and are inseparable by any means known to the bacteriologist of the present time. The condition as found leads to the belief that the root hairs of the cowpea are more penetrable by the soy bean nodule organism than are those of soy bean by the cowpea organism.

TABLE 1

Evidence of a cross inoculation relationship between the cowpea and soy bean strains of B. radicicola

CULTURE NUMBER	SOURCE OF B. RADICICOLA	AGE	NODULE PRODUCTION ON			
			Barchet soybean		Brabham cowpea	
			Total nodules	Average per plant	Total nodules	Average per plant
		years				
Control			0 −54*	0	0 −13*	0
179	Lima Bean	9.25	0 −25	0	31 −13	3−
182	Brabham cowpea	9.25	1 −13	0	47 −14	3+
185	Cowpea variety	9.25	0 −20	0	30 −12	3−
213	Adzuki bean	7.75	9 −19	1−	18 −9	2
216	Groit cowpea	7.75	7 −14	1−	35 −14	2+
220	Beggarweed	7.75	0 −22	0	41 −12	3+
243	Lima bean	7.75	0 −25	0	27 −14	2−
277	Groit cowpea	6.75	0 −16	0	56 −14	4
278	New Era cowpea	6.75	10 −15	1−	49 −15	3+
294	Japan clover	5.75	0 −19	0	37 −12	3+
300	Tepary bean	6.75	17 −20	1	42 −7	6
309	New Era cowpea	5.75	0 −23	0	26 −12	2+
313	Furze	4.75	73 −16	5−	26 −18	1+
316	Moth bean	4.75	1 −23	0	18 −9	2
321	Adzuki bean	4.75	19 −15	1+	33 −11	3
322	Velvet bean	3.75	0 −21	0	47 −10	5−
323	Velvet bean	3.75	0 −18	0	24 −12	2
324	Moth bean	3.75	23 −19	1+	33 −13	3−
325	Mung bean	3.75	0 −22	0	29 −13	2+
326	Brabham cowpea	3.75	9 −10	1−	37 −15	2+
333	Pigeon pea	2.75	0 −21	0	8 −3	3−
348	Tepary bean	2.75	3 −12	1−	18 −16	1+
349	Brabham cowpea	2.75	0 −26	0	45 −13	3+
351	*Acacia melanoxylon*	2.75	0 −18	0	29 −15	2−
354	Lyon bean	2.75	0 −18	0	30 −12	2+
369	*Cassia nictitans*	2.75	56 −20	3−	41 −12	3+
376†	Cowpea variety	?	29 −29	1	61 −24	3−
432‡	Cowpea variety	?	2 −14	1−	22 −3	7+
434§	Cowpea variety	?	18 −25	1−	10 −4	2+
180	Soy bean variety	9.25	26 −12	2+	15 −13	1+
181	Roosevelt soy bean	9.25	74 −18	4+	27 −12	2+
187	Roosevelt soy bean	9	48 −24	2	29 −16	2−

* Total number of nodules is given by first figure and number of plants by the figure following the dash.

† Culture furnished by Dr. A. L. Whiting, University of Ill. Urbana, Ill.

‡ Cultures furnished by Dr. W. A. Albrecht, University of Missouri, Columbia, Mo.

§ Cultures furnished by Laboratory of Plant Physiology, N. Y. State College of Agriculture, Ithaca, N. Y.

TABLE 1—*Continued*

CULTURE NUMBER	COURSE OF B. RADICICOLA	AGE	NODULE PRODUCTION ON			
			Barchet soybean		Brabham cowpea	
			Total nodules	Average per plant	Total nodules	Average per plant
		years				
218	Barchet soy bean	7.75	65 −17	4−	12 −14	1−
255	Virginia soy bean	6.75	40 −10	4	15 −8	2−
256	Taha soy bean	6.75	50 −28	2−	24 −10	2+
258	Tokio soy bean	6.75	116 −31	4−	23 −9	3−
270	Cloud soy bean	6.75	12 −7	2−	31 −15	2+
272	Arlington soy bean	6.75	55 −21	3−	27 −18	2−
273	Hope soy bean	6.75	40 −19	2+	22 −15	2−
275	Pingsu soy bean	6.75	33 −20	1+	27 −10	3−
289	Chinese soy bean	6	47 −20	2+	21 −8	3−
293	Barchet soy bean	5.75	51 −20	3−	18 −11	2−
310	Barchet soy bean	5.75	41 −14	3−	16 −11	1+
311	Chinese soy bean	4.75	51 −18	3−	17 −14	1+
328	Barchet soy bean	3.75	41 −13	3+	16 −8	2
329	Chinese soy bean	3.75	42 −17	2+	13 −13	1
334	Haberlandt soy bean	2.75	49 −20	2+	18 −15	1+
335	Chinese soy bean	2.75	55 −21	3−	15 −11	1+
336	Virginia soy bean	2.75	73 −18	4+	20 −10	?
399	Wilson soy bean	1.25	46 −24	?+	22 −17	1+
400	Wilson soy bean	1.25	48 −22	2+	26 −10	3−
403	Guelph soy bean	0.75	52 −23	2+	15 −9	2−
429‖	Soy bean variety	6	66 −19	3+	90 −15	6
430¶	Manchu soy bean	?	7 −7	1	2 −2	1
431‡	Soy bean variety	?	13 −19	1−	20 −16	1+
433§	Soy bean variety	?	45 −17	2+	14 −10	1+

¶ Culture furnished by Dr. E. B. Fred, University of Wisconsin, Madison, Wisconsin.
‖ Culture of Prof. Roy Hansen furnished by Dr. F. Löhnis, U. S. Dept. of Agriculture.

Indeed the data presented and the results of field observations by other investigators gives the impression that the relationship is quite one sided. Work is in progress to determine whether bacteria from nodules produced on cowpea plants by soy bean bacteria possess more tendency to infect the root hairs of the soy bean than cultures of cowpea organisms from normally produced nodules, and other questions concerning this subject which have arisen during the course of this preliminary study.

SUMMARY

An interchangeability of the nodule-forming function between cultures of *B. radicicola* from soy bean *Soja max* and cowpea *Vigna sinensis* is indicated by work carried out under pure culture conditions.

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THE RELATION OF SEED WEIGHT TO THE GROWTH OF BUCKWHEAT IN CULTURE SOLUTION[1]

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During the past year a series of experiments dealing with the relation of seed weight to germination, subsequent growth of the plants, and crop production was begun. It is the purpose of this paper to report briefly and in a preliminary way the results of a portion of this work which had to do with the growth of buckwheat plants (*Fagopyrum esculentum* Moensch.) in culture solutions under experimental conditions, with respect to the environmental complex, which was approximately the same for all the plants.

It has long been recognized, of course, that the early growth and the subsequent development of plants may be greatly influenced by the amount and quality of the food materials stored in the seed. However, the general principles underlying and governing this relation are not at all well understood.

It is not the purpose here to consider uniformity in the weight of seeds in relation to the degree of variability of the plants grown from them but merely to study, from the standpoint of several quantitative plant measurements, the growth rates of the plants as these are influenced by the weight of the seeds from which they are grown. It is interesting to note, however, that the results obtained do indicate very definitely that plants grown from seeds selected for uniformity in weight show markedly less variability than do similar plants grown from seeds not so carefully selected.

It is a pleasure here to acknowledge grateful thanks to Dr. J. W. Shive for valuable suggestions, criticism, and aid in planning and executing the work, and to the various members of the department staff for helpful and timely aid during the progress of the investigation.

METHODS OF PROCEDURE

Seeds of a commercial strain of Japanese buckwheat were weighed accurately to the tenth of a milligram. Of these, five weight-grades of seeds were selected as follows: (1) 40.5 mgm., (2) 35.5 mgm., (3) 32.5 mgm., (4) 29.5 mgm., and (5) 23.5 mgm. No seed was used which varied more than 0.4 mgm. from the weight grade value. The seed selected for each particular grade were germinated in grade-groups on a germinating net as described

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by Shive (4) and from the relatively large number of seeds germinated in each group five seedlings selected for uniformity of size and vigor were chosen for the experiment.

When the cotyledons had fully opened the seedlings were transferred to the culture vessels which consisted of bottles having a capacity of approximately 1050 cc. The neck of each bottle which was about 4 cm. in diameter, inside measurement, was file-marked. Each bottle was fitted with a paraffined cork stopper and marked with the number of the plant assigned to it and with the seed-weight grade from which the seedling was selected. Each bottle with the cork stopper was carefully weighed empty and again when filled to the file-mark with solution at a temperature of 21°C., all such weights being taken carefully to the tenth of a gram on a torsion balance.

For the early physiological growth period extending from germination to the flowering stage, Shive's three-salt solution R_4C_2 was used. This solution has been found well adapted for the growth of buckwheat (5) and contained the three salts KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$ in concentrations of 0.0144 m., 0.0052 m., and 0.0200 m., respectively. To each liter of solution used, 0.5 mgm. of iron in the form of a freshly prepared solution of ferrous sulfate was added.

After the seedlings were transferred to the culture solutions the cultures were placed on a rotating table in order to insure to all the plants similar environmental conditions. At the end of each 3.5-day interval throughout the growth period the cultures were removed from the rotating table, the old solution discarded, and the bottles again filled to the file-mark with new solution at 21°C. The cultures were then weighed and returned to the rotating table.

At the end of the seventh growth interval when the plants were in full bloom, Shive's three-salt solution R_3C_5 was substituted for that used during the early growth stage. This change is necessary, according to Shive and Martin (6), in order to produce optimum growth of buckwheat plants in these three-salt solutions during the period from the flowering stage to maturity.

The plants were harvested after they had been in culture 42 days and the dry weight of tops and roots and the leaf area of each plant were obtained separately. The leaf areas were secured by blue-printing the fresh leaves and subsequently determining the areas of these leaf-prints for each plant by means of the planimeter.

An attempt was here made to obtain the total green weight (tops and roots) of each plant at the end of the various growth intervals throughout the entire growth period. At the end of the growth intervals each plant was removed from its culture bottle together with the cork stopper in which it was mounted, placed in an empty container and allowed to drain thoroughly. The old solution was then discarded and the culture bottle filled to the file-mark with new solution at 21°C., the last few cubic centimeters of solution being added from a burette for the sake of greater accuracy. The plants were then replaced and each culture accurately weighed to the nearest tenth of a gram on a torsion balance. By deducting the weight of the filled bottle and cork stopper from the total weight the approximate green weight of the plant as well as the increase in weight from interval to interval was derived. The method is faulty in some respects. It does not take into account the weight of the solution adhering to the roots after draining, but since the error thus introduced is approximately the same for all the plants the relations indicated by the green weight data thus obtained should not be appreciably different from the true relations. Its accuracy is also decreased by variations due to transpirational fluctuations of the individual plants.

EXPERIMENTAL DATA

Green weights

In table 1 are given the total green weights of the plants as these were here derived. The first green weights were taken on the tenth day after the plants

were placed in the culture solution and at the end of each succeeding 3.5-day growth interval. The weights of the five plants and the averages of these are shown for each seed-weight grade.

TABLE 1

Green weights of plants obtained at intervals throughout the growth period

SEED-WEIGHT GRADE	WEIGHTS AFTER VARYING PERIODS OF GROWTH IN CULTURE									
	10.0 days	13.5 days	17.0 days	20.5 days	24.0 days	27.5 days	31.0 days	34.5 days	38.0 days	41.5 days
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
40.5 mgm.	4.0	6.0	8.0	8.6	13.0	15.5	15.0	16.5	20.0	18.2
	5.0	6.7	9.7	10.6	16.6	19.8	20.9	24.4	24.9	25.4
	5.0	5.8	7.5	7.5	12.5	14.3	15.2	16.5	17.5	16.5
	3.7	6.2	6.1	8.1	9.8	12.1	12.6	14.5	14.6	15.1
	3.0	5.5	6.8	8.6	10.6	11.6	13.4	13.6	15.6	16.0
Average	4.14	6.04	7.6	8.7	12.5	14.5	15.4	17.1	18.5	18.2
35.5 mgm.	4.0	6.2	8.0	8.0	12.0	16.0	17.0	17.4	19.6	20.8
	4.6	9.1	8.2	10.6	13.1	15.6	15.8	16.6	16.8	18.3
	4.4	7.3	8.4	9.6	11.6	15.1	16.4	17.6	18.1	20.4
	4.2	7.0	8.5	10.5	14.5	17.0	17.5	20.7	21.7	23.0
	3.8	6.3	8.3	10.1	13.1	15.3	14.9	16.6	17.6	18.6
Average	4.2	7.18	8.3	9.76	12.86	15.8	16.3	17.8	18.76	20.26
32.5 mgm.	4.2	7.0	8.0	8.7	12.7	14.2	14.7	15.9	16.4	17.2
	5.0	6.2	6.6	7.8	12.1	14.6	16.6	18.6	19.3	20.1
	3.0	5.4	7.0	6.2	10.7	14.4	14.2	15.7	17.2	17.6
	3.2	5.0	6.7	6.9	10.4	11.9	13.7	14.7	15.9	15.4
	4.6	6.9	8.1	10.5	14.9	16.9	16.9	18.4	19.7	18.9
Average	4.0	6.1	7.3	8.02	12.16	14.4	15.2	16.66	17.7	17.84
29.5 mgm.	5.3	6.8	9.3	10.3	13.4	16.1	18.1	18.8	20.3	19.8
	3.1	5.6	6.8	6.8	11.8	13.8	15.0	17.8	18.2	17.8
	3.0	5.5	7.2	7.7	11.3	11.9	13.7	14.7	15.3	14.7
	4.3	5.8	7.3	8.8	12.5	14.3	15.8	17.8	18.3	17.8
	3.5	6.5	8.3	8.8	12.3	14.8	15.8	16.8	16.3	17.8
Average	3.94	6.04	7.8	8.7	12.26	14.2	15.7	17.2	17.7	17.58
23.5 mgm.	3.5	5.2	6.5	6.8	10.3	12.3	14.3	15.4	16.8	17.3
	2.7	5.5	6.3	6.3	10.2	12.8	13.3	14.8	15.6	16.8
	3.0	5.4	6.8	8.4	12.9	14.1	16.3	17.1	17.1	17.7
	3.0	3.5	6.8	6.9	10.1	11.4	13.4	14.0	14.9	13.4
	4.2	6.8	8.2	9.5	13.3	15.5	16.5	18.8	21.5	21.3
Average	3.7	5.3	6.9	7.6	11.36	13.3	14.76	16.04	17.2	17.3

From the data of table 1 it will be observed that the green weight averages for the plants grown from the smallest seeds (23.5 mgm. grade) are the lowest throughout, while those from the next to the largest seeds (35.5 mgm. grade) are the highest throughout and that the order of superiority of the plants grown

from seeds of different weight corresponds to the order of seed wieght from the lowest to the next to the highest here used. The plants grown from the abnormally large seeds (40.5 mgm. grade) were slightly inferior in average green weight throughout to those grown from the large medium seeds (35.5 mgm. grade).

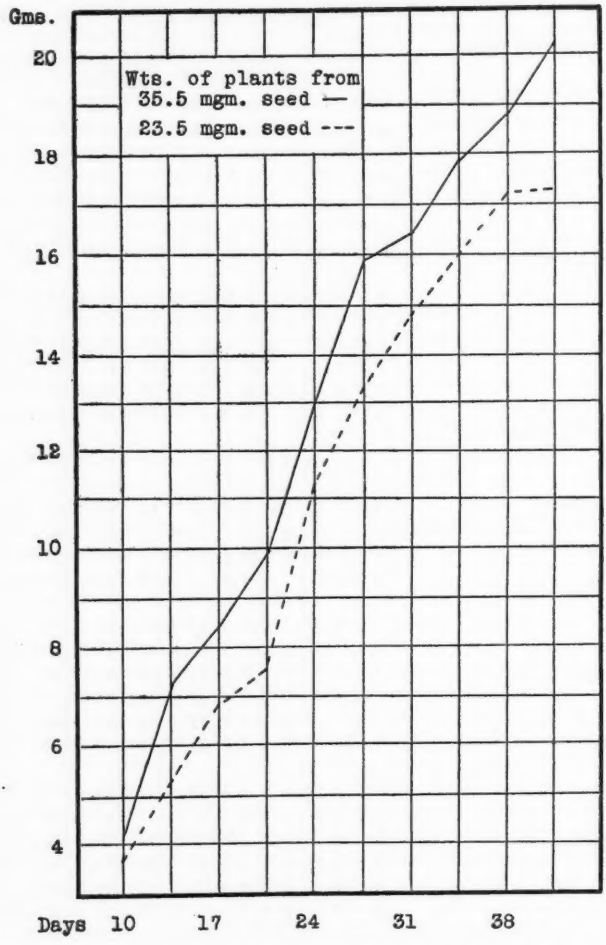

FIG. 1. GRAPHS OF AVERAGE GREEN WEIGHTS OF PLANTS GROWN IN SOLUTION CULTURES FROM SEEDS WHICH PRODUCED THE HIGHEST AND LOWEST YIELDS

The average green weight data for the plants grown from the grades of seeds which produced the highest and the lowest yields are plotted to form the graphs of figure 1. The graphs representing the average green weight data corre-

sponding to the remaining three seed-weight grades are here omitted since they occupy positions intermediate between the two graphs shown in figure 1, and do not intersect them at any point.

TABLE 2

Dry weights and leaf areas of plants grown in culture solutions

SEED-WEIGHT GRADE	DRY WEIGHTS			LEAF AREAS
	Tops	Roots	Total	
	gm.	*gm.*	*gm.*	*sq. cm.*
40.5 mgm.	1.6615	0.1061	1.7676	223.1
	2.0950	0.1677	2.2627	312.2
	1.5546	0.0918	1.6463	225.8
	1.2300	0.0728	1.3028	202.1
	1.1257	0.1151	1.2408	181.1
Average	1.5333	0.1107	1.6440	228.9
35.5 mgm.	1.4615	0.1344	1.5959	241.4
	1.6035	0.1067	1.7092	235.8
	1.6004	0.1205	1.7209	241.4
	1.7793	0.1040	1.8833	221.7
	1.5026	0.1547	1.6573	254.9
Average	1.5895	0.1239	1.7133	239.0
32.5 mgm.	1.4425	0.0617	1.5042	212.8
	1.4842	0.1250	1.6092	226.9
	1.6840	0.1271	1.8111	243.6
	1.3522	0.1224	1.4746	221.2
	1.7814	0.1066	1.8880	245.0
Average	1.5489	0.1086	1.6574	229.9
29.5 mgm.	1.5057	0.1327	1.6384	231.8
	1.6638	0.1164	1.7802	236.9
	1.4952	0.1208	1.6160	231.5
	1.4652	0.1294	1.5946	227.0
	1.2745	0.1175	1.3920	170.7
Average	1.4809	0.1234	1.6042	219.6
23.5 mgm.	1.3587	0.0946	1.4533	202.6
	1.2040	0.0982	1.3022	182.6
	1.3590	0.1267	1.4857	195.3
	0.9260	0.0909	1.0169	147.5
	1.7540	0.1561	1.9101	272.9
Average	1.3163	0.1133	1.4336	200.2

It is clearly apparent from the graphs of figure 1 that the advantage, with respect to green weights, in favor of the plants grown from the heavier seeds over those from the lighter seeds is not only maintained throughout the entire growth period but is also gradually, though not very greatly, augmented as the plants become older. The average green weights of the plants grown from

the heavier seeds were, at the end of the first and last growth intervals represented on the graphs, 13.1 per cent and 17.1 per cent heavier, respectively, than were the corresponding weights of the plants grown from the lightest seeds employed.

Dry weights and leaf areas

The absolute dry weight yields and the leaf areas of the mature plants together with the averages of these plant measurements corresponding to each seed-weight grade are presented in table 2. The average dry weight yields of

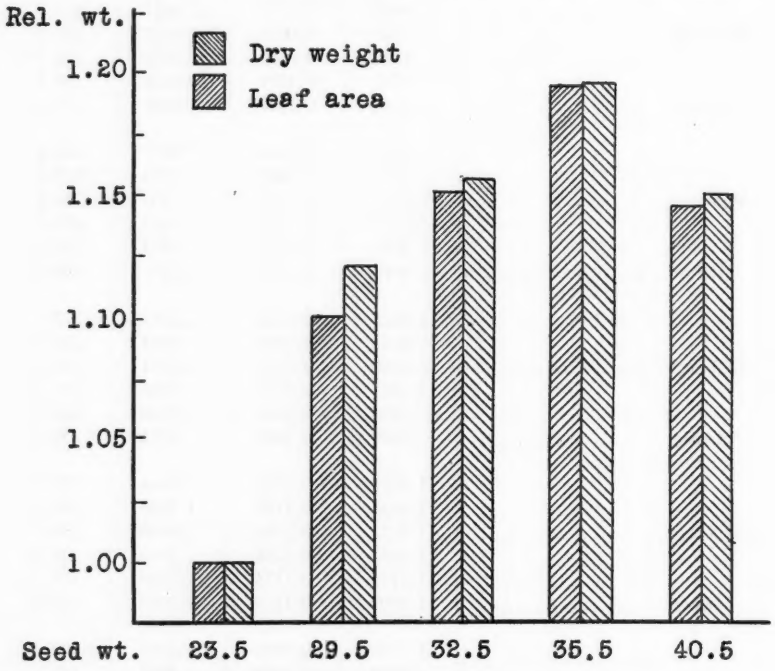

FIG. 2. DIAGRAM COMPARING AVERAGE RELATIVE DRY WEIGHTS WITH AVERAGE RELATIVE LEAF AREAS OF PLANTS GROWN FROM SEEDS OF DIFFERENT WEIGHTS

tops and the average leaf areas are shown diagrammatically in comparison in figure 2, but all the values are here expressed in terms of those corresponding to the lowest seed-weight grade (23.5 mgm.) taken as 1.00.

As is clearly brought out by the diagram of figure 2, the order of superiority of the plants grown from the seeds of different weights, with respect to average dry weights of tops, average total dry weights, and average leaf areas, corresponds to the order of seed weights from the lowest to the next to the highest,

this relation being, therefore, in absolute agreement with that of the green weight values. No such relation, however, exists between seed weights and dry weights of roots, although the seed-weight grade corresponding to the highest average yield of tops, total yield, and leaf area corresponds also to the highest dry weight of roots. The average weight of tops, total dry weight, and leaf area obtained with the seeds from the highest yielding weight-grade (35.5 mgm.) are 20.8 per cent, 19.5 per cent, and 19.4 per cent higher, respectively, than are the corresponding yield values obtained with the seeds from the lowest weight-grade (23.5 mgm.).

It is interesting and important to note that the average leaf areas are nearly proportional to the average dry-weight yields of tops, as is apparent from the diagram of figure 2 and from the data of table 2. This is in entire accord with the work of McLean (3) and Hildebrandt (1, 2) who found that the leaf areas of soybean plants at the age of four weeks are approximately proportional to the dry weight of stems and leaves. Since, as is clear from the data of table 2, the total dry weights of the buckwheat plants here used are approximately proportional to the dry weights of tops, this relation holds also between total dry weight yields (tops and roots) and leaf areas. On the other hand, no such relation is apparent between dry weights of roots and leaf areas. This follows, of course, since there is no definite correlation between the growth of tops and roots of the buckwheat plants here employed.

It is to be emphasized, of course, that the data here presented are too meager and inadequate to justify anything more than suggestion. Definite conclusions are not warranted and the purpose of the paper has been achieved by a brief consideration of some of the more important points which the data have conveyed.

SUMMARY

Buckwheat plants were grown in solution cultures from seeds of five different weight-grades, under experimental conditions which were approximately alike for all the plants.

1. Seeds of high medium weight produced better plants, from the standpoint of several quantitative plant measurements (averages only considered), than did seeds of lighter weight or abnormally heavy seeds.

2. The order of superiority of the plants grown from the heavier seeds over those grown from seeds of lighter weight corresponds to the order of seed-weight, except for the abnormally heavy seeds.

3. Under conditions which were approximately the same for all plants, the superiority of those grown from heavier seeds over those grown from lighter seeds was maintained from the early seedling phase to maturity.

4. Leaf areas were approximately proportional to dry weights of tops and to total dry weights but no such relation was apparent between dry weights of roots and leaf areas.

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SOLUBILITY OF LIMESTONES AS RELATED TO THEIR PHYSICAL PROPERTIES

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INTRODUCTION

Limestone varies widely in mineral structure and chemical composition. Its extensive use in agriculture in the correction of soil acidity creates a need for some accurate basis for the establishment of its value for this purpose. The total neutralizing power is commonly accepted as an absolute measure of agricultural value. From an economic standpoint however, the farmer is primarily interested in the rate of reaction of the limestone, since upon this factor depends the rapidity with which the undesirable conditions due to acidity will be corrected.

The effects of fineness of grinding and of chemical composition upon the rate of solubility of limestone have been studied in detail by several workers, notable Frear (5), Ames and Schollenberger (1), Barker and Collison (2), and Stewart and Wyatt (9). No experimental data has yet been published showing the influence of physial properties such as porosity, hardness, specific gravity and crystalline structure upon the rate of reaction.

Frear (4, p. 175) states that it is entirely probable that a porous, impure limestone will break down more rapidly than a compact, pure stone because such is the case with massive rocks showing similar differences in texture and purity. In a later publication (5) he adds that the increased solubility of porous stone may not be as great as otherwise expected, due to the relatively much smaller solubility in the capillary pores than on the free surface.

Lacking definite information upon this subject, popular opinion has been that the crushed or ground product from soft, porous limestones is more rapidly soluble than the material from hard, compact rocks of similar chemical composition. In an effort to throw some light upon this subject, the authors have investigated the solubility of a series of limestones showing marked differences in their physical properties.

EXPERIMENTAL

A series of twelve limestone materials, varying widely in their physical and chemical nature and described in tables 1, 2 and 3 were selected for this study. Solubility was determined by measuring the rate of reaction with dilute standard acetic acid (table 4), the decrease in lime requirement of acid soils produced

TABLE 1

Descriptive data on limestones

NUMBER	SOURCE	FORMATION	HARDNESS	POROSITY	STRUCTURE	COLOR	COMPOSITION
1	Martinsburg, W. Va.	Stone's River	Very hard	Very compact	Very fine grained non-crystalline	Dove	High-calcium moderate purity
2	Muskingum Co., Ohio	Maxville	Very hard	Compact	Fine grained non-crystalline	Blue-gray	High calcium
3	Fremont, Ohio	Monroe	Medium	Medium	Medium grained non-crystalline	Drab	Dolomite moderate purity
4	Youngstown, Ohio	Putnam Hill	Very hard	Compact	Coarse grained non-crystalline	Dark blue-gray	High calcium slightly impure
5	Piqua, Ohio	Brassfield	Medium	Medium	Coarsely crystalline	Light gray	Slightly dolomitic
6	Piqua, Ohio	Brassfield	Medium soft	Medium	Very crystalline	White	Moderately dolomitic
7	Kelly Island, Ohio	Columbus	Medium soft	Medium	Coarse grained non-crystalline	Gray	Moderately dolomitic
8	Port-of-Calcite, Mich.	Dundee	Soft	Porous	Medium grained non-crystalline	Light gray	Very pure high calcium
9	Carey, Ohio	Niagara	Medium hard	Very porous	Very crystalline	White	Pure dolomitic
10	Yellow Springs, Ohio	Springfield	Hard	Very porous	Coarse, partly crystalline	Rusty gray	Dolomite
11	Yellow Springs, Ohio	Travertine	Very soft	Very porous	Coarse, loose non-crystalline	Yellowish gray	High calcium
12	New Hampshire	Marble	Soft	Very compact	Medium grained very crystalline	White	High calcium

by the several materials at the end of varying periods of time (table 5) and the diminution of the carbonate content produced during a given period of exposure to acid soil conditions (table 6).

TABLE 2

Results of physical tests

NUMBER	PORE-SPACE IN TOTAL VOLUME	SPECIFIC GRAVITY	HYGROSCOPIC CAPACITY		ABRASION IN 2 HOURS
			50 mesh	100 mesh	
	per cent		*per cent*	*per cent*	*per cent of initial weight*
1	0.16	2.717	0.098	0.110	6.00
2	1.46	2.751	0.454	0.456	6.25
3	9.23	2.823	0.138	0.175	7.10
4	0.48	2.718	0.200	0.250	5.65
5	4.43	2.759	0.195	0.185	7.90
6	0.49	2.756	0.054	0.060	9.15
7	14.10	2.769	0.062	0.078	11.25
8	13.40	2.653	0.070	0.087	11.65
9	18.21	2.781	0.185	0.188	8.30
10	17.62	2.790	0.216	0.269	7.60
11	45.00	2.465	0.440	0.458	39.45
12	0.39	2.723	0.042	0.060	28.20

TABLE 3

Chemical analyses

NUMBER	$CaCO_3$	$MgCO_3$	SiO_2	Fe_2O_3	OTHER OXIDES	NEUTRALIZING POWER $CaCO_3$=100
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	
1	86.30	1.74	9.81	1.41	1.68	88.37
2	89.80	1.98	3.86	1.44	1.15	92.16
3	46.70	38.86	8.06	0.59	5.91	92.91
4	76.20	1.32	14.42	2.99	3.71	77.77
5	81.30	7.68	5.42	1.92	1.70	90.45
6	82.80	12.39	2.91	0.86	0.55	97.55
7	79.60	13.85	4.13	1.01	1.52	96.10
8	96.20	1.17	0.86	0.38	0.14	97.60
9	54.10	44.00	0.74	0.36	0.38	106.50
10	51.80	42.17	1.36	1.74	2.95	101.80
11	95.80	1.77	0.99	0.18	0.11	97.90
12	96.40	0.86	2.20	0.32	0.31	97.40

In these investigations porosity en masse and specific gravity were determined by the method outlined by Hillebrand (6, p. 51–53). Hygroscopic capacity was taken as a measure of the porosity of the finely ground material. It was determined by drying the sample to constant weight in an oven at 110°C. and determining the amount of water absorbed when placed over 10-per-cent H_2SO_4 for two weeks.

Hardness was determined by measuring the abrasion which took place when particles between 3- and 4-mesh in size were rotated in a pebble mill; 100 gm. of material were used, with rotation in the mill for 2 hours. At the end of this period the fraction which passed a 10-mesh screen was determined.

TABLE 4

Solubility of limestone materials in 2 N acetic acid

NUMBER	AMOUNT REACTING IN 30 MINUTES	
	50 mesh	100 mesh
	per cent	*per cent*
1	71.4	79.6
2	64.6	76.0
3	10.4	11.2
4	63.8	77.6
5	57.6	66.6
6	50.8	52.4
7	51.0	62.4
8	60.8	75.0
9	6.4	7.6
10	6.2	8.2
11	50.0	61.0
12	48.0	50.6

TABLE 5

Effect of application of limestone materials upon $CaCO_3$ requirement of acid soils, per 2,000,000 pounds

NUMBER	TRUMBULL SILT LOAM			DEKALB SANDY LOAM	
	4th day	11th day	35th day	6th day	20th day
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*
50-mesh separate					
1	1812	1561	1500	2937	656
2	1750	1125	937	2531	625
3	2437	2250	2125	4656	2906
4	1787	1287	1000	2906	812
5	2031	1812	1625	3469	1219
6	2375	1825	1656	4219	2062
7	2312	2000	1750	4190	2000
8	2000	1625	1500	3000	1125
9	2406	2375	2310	5125	3562
10	2406	2394	2250	5000	3219
11	1781	1337	1062	2781	875
12	2194	1938	1781	3594	1375
100-mesh separate					
1	1094	781	375	750	281
2	844	562	190	656	220
3	2187	1812	1060	2812	1375
4	969	656	250	844	406
5	1375	937	625	1594	437
6	1812	1312	750	2375	656
7	1687	1125	689	1937	625
8	1594	912	437	1469	375
9	2219	1842	1125	3500	1669
10	1919	1594	1060	3031	1562
11	1500	719	406	1125	437
12	1562	1062	562	1531	500

In the solubility investigations two sizes of material were used; first, that which passed a 40-mesh and was retained by a 50-mesh screen; second, that which passed an 80-mesh and was retained by a 100-mesh screen. These separates were thoroughly washed on the screen to insure freedom from finer material.

It was believed that the relative solubilities of the various materials could be satisfactorily determined by measuring their rate of reaction in an excess of dilute acetic acid of standard strength during a definite time interval. Acetic acid was chosen on account of its low dis-

TABLE 6

Residual carbonates calculated as $CaCO_3$ after application of limestone materials to acid soils

NUMBER	$CaCO_3$ PER 2,000,000 LBS. SOIL			
	Trumbull silt loam—35th day		DeKalb sandy loam—20th day	
	Residual	Neutralized	Residual	Neutralized
	lbs.	*lbs.*	*lbs.*	*lbs.*
50-mesh separate				
1	6430	3570	9040	10960
2	5570	4430	8860	11140
3	7950	2050	13880	6120
4	6540	3460	9590	10410
5	7270	2730	10340	9660
6	7640	2360	12070	7930
7	7390	2610	12160	7840
8	6640	3360	10180	9820
9	8290	1710	14430	5570
10	8130	1870	14270	5730
11	6090	3910	9950	10050
12	6920	3180	10470	9630
100-mesh separate				
1	3140	6860	7620	12380
2	2930	7070	6460	13540
3	5040	5960	10320	9680
4	3000	7000	7230	12770
5	4090	5910	7960	12040
6	4630	5370	8320	11680
7	4160	5840	8390	11610
8	3250	6750	7680	12320
9	5220	4780	11160	8840
10	5090	4910	10980	9020
11	3020	6980	7350	12650
12	4090	5910	8000	12000

sociation ($K = 1.8 \times 10^{-5}$), which permits considerable change in the total acid present without altering appreciably the hydrogen-ion concentration of the solution; it was desirable in this work to keep this factor as nearly constant as possible.

Method employed: A sample of limestone containing the equivalent of 5 gm. of $CaCO_3$ was placed in a 300-cc. Erlenmeyer flask, 50 cc. of 2 *N* acetic acid were added and the flask was kept in constant agitation for 30 minutes to avoid irregularities of diffusion. At the end of this period the liquid in the flask was filtered as quickly as possible, and a 5-cc. aliquot titrated with 0.2 *N* NaOH, using bromthymol-blue as the indicator. From this titration

the amount of limestone reacting with the acid during the period was calculated. The change in concentration of the acid was less than pH 1, as determined colorimetrically.

To determine the solubility of the limestone particles when added to an acid soil, two soils were employed, a Trumbull silt loam and a DeKalb silt loam, having limestone requirements of 3,200 and 6,100 lbs. respectively. A modification of the Jones lime requirement method (7) was used, wherein bromthymol-blue was substituted for phenolphthalein as the indicator, and the $CaCO_3$ requirement was calculated directly from the titration, rather than by using the arbitrary factor of 1.8. To insure a sufficient excess of limestone to neutralize all the acidity present, applications were made to the soils at rates equivalent to 10,000 and 20,000 lbs. of $CaCO_3$ per 2,000,000 lbs. of soil. The limestone particles were thoroughly mixed with the dry soil and sufficient water was added to obtain the optimum moisture-content. It was found that the progress of the reaction could be observed by determining the $CaCO_3$ requirement by the foregoing method. A rubber pestle was used in mixing of the soil with the calcium acetate to prevent mechanical disintegration of the limestone particles. As a check upon the results thus obtained, the Truog (10) test was used. A very close correlation was found between the results of the Jones and Truog methods.

RATE OF SOLUBILITY IN ACID SOILS, AS MEASURED BY RESIDUAL CARBONATES

In the decomposition of limestone in an acid soil, the carbonates are, presumably, neutralized as rapidly as they go into solution. As pointed out by McIntire (8) the calcium and magnesium, more especially the latter, may react with the more readily soluble soil silicates with the formation of the corresponding alkaline earth silicates. In either case, a determination of the carbonates remaining in the soil at the end of a given time interval after treatment should afford a measure of the rate of solubility of the limestone materials under investigation.

With this idea in mind, residual carbonates were determined in the soils treated as previously described. A modification of Bear and Salter's (3, p. 21–22) method was used, whereby the carbonates were decomposed by treatment with strong acid, and the CO_2 liberated was absorbed by soda-lime contained in a Fleming absorption bulb. With the large quantity of soil used (20 gms.) this method proved to be more satisfactory than the volumetric procedure involving the absorption of the gas in a NaOH solution. Results are given in table 6.

DISCUSSION OF RESULTS

The limestone materials studied varied considerably in chemical composition and physical properties. It should be noted that nine of the twelve samples were from quarries producing agricultural ground limestone, so that any significant differences in their rates of solubility should be of special importance.

In a study of the data, the slower rate of reaction of the dolomitic materials is the most noticeable feature. This is clearly shown in table 4 where it is seen that the three typical dolomites no. 3, 9 and 10, are very slowly soluble in 2 *N* acetic acid, having an initial hydrogen-ion concentration of pH. 3.2

comparable to a very acid soil, while the high-calcium limestones, even though very hard and compact, were readily soluble. In the same table it is of interest to note that with both sizes of separates no. 11, a soft travertine, was much more slowly dissolved by the acid, then were nos. 1, 2 and 4, all of which were hard and compact high-calcium limestones.

Figures 1 and 2 shows in graphic form the results of the treatment of the DeKalb soil with 50 and 100-mesh material. A study of these graphs, in connection with results shown in table 5 disclose the following relationships:

1. There is a fairly close agreement in the order of solubility of the limestones for the different sized separates, at different intervals of time after application, and with the two different soils used. This order of solubility is in close agreement with that found in acetic acid, as shown in table 4.

2. In general, the rate of solubility seems to be dependent upon the relative percentages of magnesium and calcium carbonates in the stone. The six stones containing no appreciable amount of $MgCO_3$ are the more effective in decreasing the $CaCO_3$ requirement, while the three dolomites are in every case much slower in their action.

3. With corresponding applications of limestone material, the rate of reaction is much more rapid in the case of the more acid DeKalb sandy loam. This is probably due to differences in the initial hydrogen-ion concentration of the soil solutions. Prior to treatment, the Trumbull silt loam showed a reaction of pH 5.6 while that of the DeKalb sandy loam was pH 4.2.

4. The 100-mesh material brought about a more rapid rate of decrease in acidity in both soils used. The factor of fineness of grinding is seen to be of decided importance in overcoming the differences in solubility due to the nature of the stone. As applied to the Trumbull silt loam, the solubility of the 50-mesh dolomitic material is very slow and becomes much less rapid with increasing time. The 100-mesh high-calcium material, added to the same soil, reacts much more slowly as the neutral point is approached, while the high-magnesium stones of the same degree of fineness continue to reduce the $CaCO_3$ requirement at an almost uniform rate. When the materials are applied to the very acid DeKalb sandy loam, a more rapid chemical disintegration of the particles takes place, and the differences in solubility of the materials used are of a smaller order of magnitude. The 100-mesh particles of all the stones tended to bring the reaction rapidly toward the neutral point. Only for the three dolomites is there any significant $CaCO_3$ requirement remaining at the end of the 20-day period.

5. With the exception of the coarser separate of dolomitic material when applied to the less acid soil, the rate of reaction in all cases is fairly rapid. Thus in 20 days the 100-mesh material reduced the $CaCO_3$ requirement of the DeKalb sandy loam to one-twelfth the original amount.

Figure 3 shows graphically a comparison between the differences in solubility of the limestone materials used and their physical and chemical properties. The adverse effect of relatively large amounts of $MgCO_3$ upon the rate of solubility is clearly shown. Further than this it would seem impossible to draw any conclusions. The following examples of irregularity are significant: no. 2, a high-calcium stone, very hard and only slightly porous in the mass, is the most rapidly soluble in practically all the tests. Particles used seem to show a relatively high degree of hygroscopiscity, indicating a porosity in the ultimate grain rather than in the mass. On the other hand, no. 8, also a

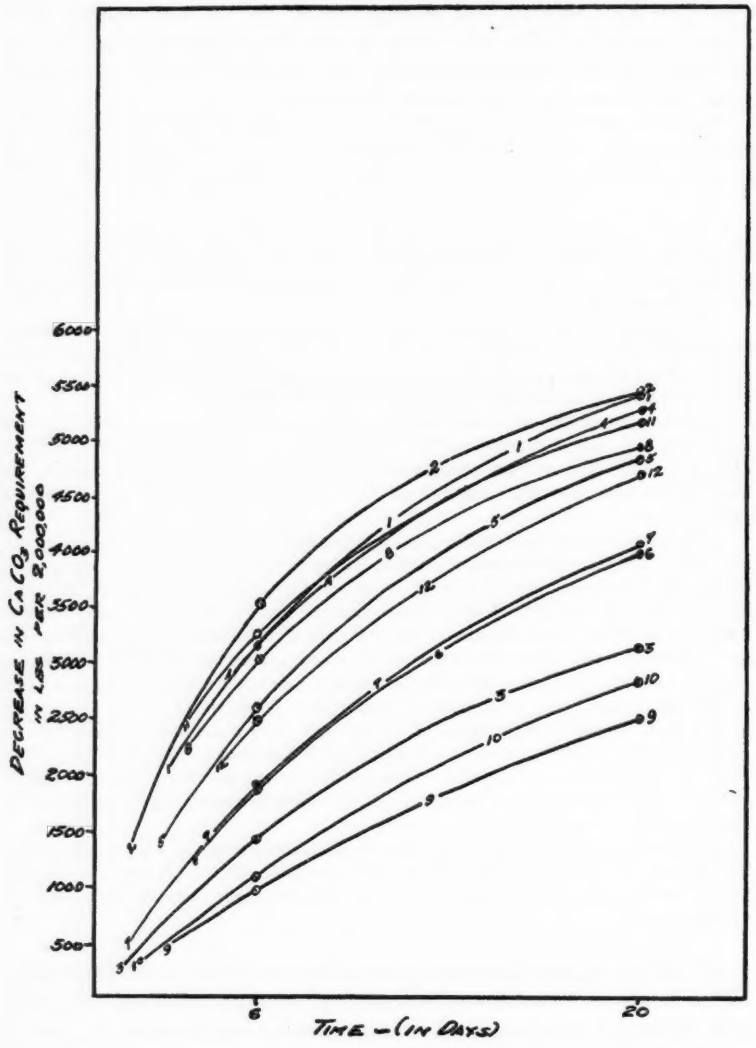

FIG. 1. EFFECT OF APPLICATION OF 50-MESH LIMESTONE MATERIALS UPON $CaCO_3$ REQUIREMENT OF ACID DEKALB SANDY LOAM

Numbers refer to limestone samples. Curves indicate effect of material in reducing the acidity at the end of a given time interval after application. Readings taken as indicated, on 6th and 20th days.

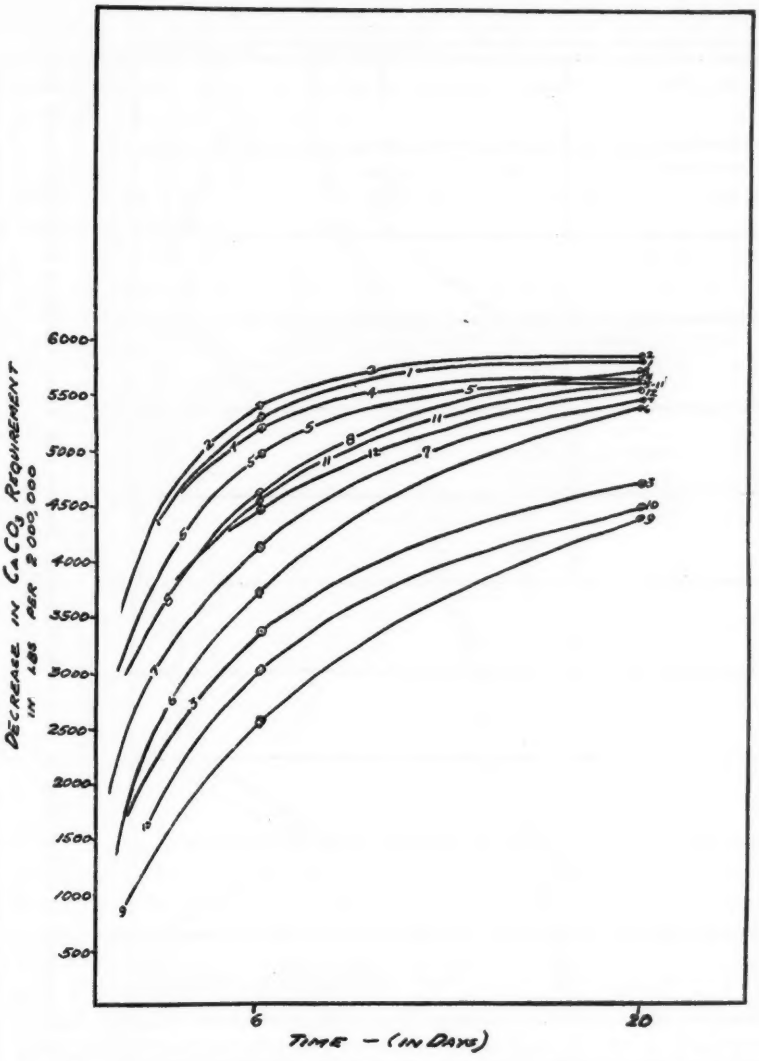

FIG. 2. EFFECT OF APPLICATION OF 100-MESH LIMESTONE MATERIALS UPON $CaCO_3$ REQUIREMENT OF ACID DEKALB SANDY LOAM

Numbers refer to limestone samples. Curves indicate effect of material in reducing the acidity at the end of a given time interval after application. Readings taken as indicated, on 6th and 20th days.

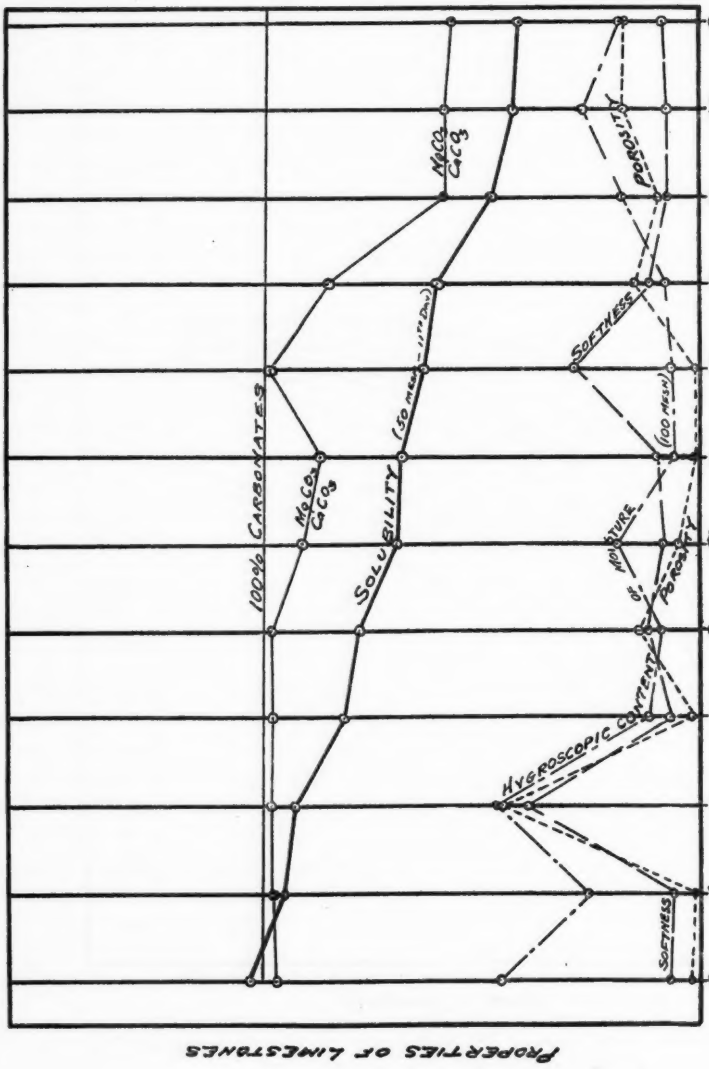

FIG. 3. COMPARISON OF SOLUBILITIES OF LIMESTONE MATERIALS STUDIED AND THEIR CHEMICAL COMPOSITION AND SOME PHYSICAL PROPERTIES

Numbers refer to limestone samples. The relative proportion of $MgCO_3$ and $CaCO_3$ on the basis of 100 per cent carbonates is shown by two upper lines. Other lines are on different abscissa, depending upon unit of measurement.

high-calcium stone, is much more slowly soluble, though softer, more porous in the mass, but with a smaller hygroscopic capacity. No. 12, a crystalline marble containing fairly coarse grains of practically pure calcite, is less readily soluble than any of the high-calcium limestones, although in the rock mass it is quite easily crushed and abrased.

It is of interest, though not necessarily significant, to note that the five stones which break into crystalline fragments are among the seven stones which are the least soluble. It happens that all but one of these are high in magnesium, two being typical dolomites.

In determining the residual carbonates after the $CaCO_3$ requirement had been materially reduced a rather definite relationship was found between the $CaCO_3$ requirement obtained by direct titration in the Jones method and the decrease in carbonates. It is thus seen that there is a very constant relation-

TABLE 7

Comparison of decrease in Jones $CaCO_3$ requirement with decomposition of carbonates

NUMBER	TRUMBULL SILT LOAM (100-MESH MATERIAL) ON THE 35TH DAY		
	$CaCO_3$ equivalent decomposed	Decrease in Jones $CaCO_3$ requirement	Factor
1	6860	2875	2.38
2	7070	3000	2.35
3	4955	2140	2.31
4	7000	2950	2.36
5	5910	2575	2.29
6	5365	2450	2.31
7	5840	2511	2.32
8	6750	2763	2.44
9	4780	2075	2.30
10	4910	2140	2.29
11	6980	2794	2.41
12	5910	2638	2.24

ship between the results given by the Jones method and the absolute lime requirement, as shown by the soil's ability to liberate carbon dioxide from calcium and magnesium carbonates. Complete data show a similar relationship for all the results obtained with both separates for each soil but for the sake of brevity were not included.

It is significant that the results of other measurements have been so well verified by the determination of residual carbonates. So far as has been observed, there has been no tendency for the dolomitic limestones to be decomposed through conversion into magnesium silicate at a more rapid rate than the high-calcium limestones. These observations are apparently at variance with results reported by McIntire (8), but it should be noted that he worked with material passing a 100-mesh screen, which may have contained a large amount of very fine particles. The relatively greater solubility of the 100-

mesh separate from the dolomitic stones might indicate a tendency similar to that observed by McIntire. It must also be remembered that in the present work the neutral point was not reached and while any acidity exists, no appreciable formation of magnesium silicate would be expected.

The results relative to the slower rate of reaction of the dolomitic stones are in agreement with the findings of Ames and Schollenberger (1) and White and Gardner (11, p. 16).

CONCLUSIONS

With particles of the size ordinarily found in agricultural ground limestone, there is no apparent relationship between the rate of solubility in acid soils and any physical property of the rock material.

The rate of solubility is very largely influenced by the relative amount of $MgCO_3$ in the material. With coarse particles, the decreased solubility may be of considerable significance. The finer the material is ground, the less important this factor becomes, and with limestone containing considerable 100-mesh material, it is believed that the rate of solubility of dolomitic stones would be sufficiently rapid for all practical purposes.

SUMMARY

1. A study has been made of twelve limestone materials, covering a wide variation in physical and chemical properties, with the purpose of determining the factors influencing their rates of solubility when applied to acid soils.

2. The materials used were tested for hardness, porosity en masse, porosity of the 50- and 100-mesh separates as determined by hygroscopic capacity, specific gravity, crystalline composition and chemical analysis.

3. The rate of solubility of 50- and 100-mesh separates of the materials used was determined: first, by solubility in 2*N* acetic acid during 30 minutes; second, by measuring the limestone requirement and residual carbonates at definite intervals after their application to two soils of different degrees of acidity.

4. There was no apparent relationship between any physical properties determined and the rate of solubility.

5. The limestones used varied greatly in their rate of solubility, but showed practically the same order of solubility by all of the methods employed.

6. The differences in solubility of limestones containing appreciable amounts of $MgCO_3$ could be explained by the lower solubility of dolomite in a solution having a hydrogen-ion concentration comparable to that prevailing in an acid soil.

7. The differences in solubility of limestones containing no appreciable amount of $MgCO_3$ could not be explained with the data obtained in these investigations.

8. Further study upon differences in solubility of high-calcium limestones of similar chemical composition seems desirable.

9. The fineness to which dolomitic limestones are ground is of greater relative importance than is the case with high-calcium stones.

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A COMPARISON OF MAGNESIAN AND NON-MAGNESIAN LIMESTONE IN SOME 5-YEAR ROTATIONS[1]

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In 1908 an experiment was laid out for the purpose of testing two sources of lime, applied in different amounts and in connection with different crop rotations (3, 4). The materials used were magnesian (dolomitic) and non-magnesian limestones, each applied at the rate of 1000, 2000 and 4000 pounds per acre. For carrying out the work twenty-eight $\frac{1}{10}$-acre plots were divided into four blocks of seven plots each on which four different five-year rotations have been carried out. With the season of 1922, three 5-year periods have been completed. The rotations are as follows:

No. 1. Corn, oats, wheat, and two years of timothy and clover.

No. 2. Corn, potatoes, rye, and two years of timothy and clover.

No. 3. Corn, potatoes, tomatoes, lima beans, cucumbers. This has been called the market garden rotation.

No. 4. Corn; oats and peas, millet; rye and vetch, rape; rye or rye and vetch, cowpeas or soybeans; oats and peas, cowpeas or soybeans. This has been designated as a forage crop rotation.

The plan provides for seeding a legume cover crop between the main crops wherever possible, and it will be noted that at least one legume was introduced in each rotation. The forage crop rotation provides for at least one legume every year.

Acid phosphate and muriate of potash have been applied annually, the former at the rate of 300 to 400 pounds per acre and the latter at 100 to 200 pounds. In most cases commercial nitrogenous fertilizers were applied in amounts to furnish nitrogen equivalent to 160 to 200 pounds of nitrate of soda per acre. In some instances such as the market garden crops, heavier applications have been made. Less nitrogen has generally been used for the forage crop rotation than for the others. No farm manure has been used during the entire 15 years.

The lime requirement of this soil was not determined when the experiment was started, but determinations made on samples from the check plots at the end of the first five years, showed a requirement of approximately 1000 to 1500 pounds of lime (CaO) per acre, whereas the plots that had in 1908 received

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2000 and 4000 pounds per acre were, at the end of the first 5 years, close to the neutral point.

Careful crop records have been kept and in practically all cases samples of the dry material have been analyzed for nitrogen, so that a record might be had of the amount of nitrogen taken off the land in the form of crops.

The results of this work are conveniently shown by reporting the yields for the crops by years, with averages for the three years of each crop. In the case of corn and grain crops, the yields are reported as total dry matter in the grain and stover or straw as the case may be; the hay crops are reported as dry matter, and potatoes, tomatoes and cucumbers as marketable crops.

ROTATION 1 (PLOTS 21–27)

The results for rotation 1 are shown in table 1. It may be explained here that following the oat crop of 1909 cowpeas were sown as a cover crop but these made rather poor growth apparently for lack of thorough inoculation. Also following the wheat crop of 1910, cowpeas were seeded as a green manure crop and made fair growth before being plowed under in preparation for seeding timothy and clover. The timothy and clover was seeded in the fall of 1910 but the crop was a failure and this necessitated the seeding of oats in the spring of 1911. This explains the figures for oats in 1911 where the yields of timothy and clover should have appeared.

With very few exceptions the lime treatments resulted in some increase for each crop. In some cases this increase amounted to only a few pounds while in others it amounted to as much as 500 to 1000 pounds of dry matter per acre for the one-half ton application of limestone. In most cases the 1-ton application gave a fair increase over the half-ton application, but the 2-ton application gave either no increase or only a small increase over the 1-ton application.

Of the five crops the oats showed the least response to the lime treatment and the timothy and clover the greatest. In 1921 the plot which receives one ton of magnesian limestone yielded more than twice as much hay as the no-lime plot, and in 1922 the same plot yielded more than three times as much. In 1916 and 1922 a second cutting of hay was obtained from these plots while in 1917 and 1921 only one cutting was obtained.

Attention may be drawn to the fact that in every case one ton of magnesian limestone gave larger yields than two tons of the calcium limestone, and with slight exception as large or larger yields than the two tons of magnesian limestone.

In 11 out of 15 years one-half ton of magnesian limestone gave larger yields than one ton of calcium limestone, the general average for the former being 3721 pounds and for the latter 3449 pounds. A little figuring will show that in most cases the increases obtained with one-half ton of both forms of limestone were sufficient to make its use profitable.

Taking the five crops for three years each—15 years in all—the ½-ton application of magnesian limestone gave an average increase of approximately 1200

pounds of dry matter per acre; the 1-ton application of magnesian limestone an average increase of approximately 1400 pounds per acre and the 2-ton

TABLE 1

Yield of dry matter in crops in rotation 1 (acre basis)

YEAR	NO LIME	½ TON		1 TON		2 TONS	
		Ca limestone	Mg limestone	Ca limestone	Mg limestone	Ca limestone	Mg limestone
				Corn (grain and stover)			
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
1908	4673	4582	5842	4935	5241	5205	4846
1913	3500	4025	5350	4350	5375	4825	5200
1918	4593	4876	5271	5189	5434	5208	5721
Average	4255	4494	5488	4825	5350	5079	5256
				Oats (grain and straw)			
1909	1524	1572	2056	1560	2212	1917	2345
1914	1520	1280	1660	1640	1720	1620	1680
1919	2320	3120	3120	3080	3400	3280	3360
Average	1788	1991	2279	2093	2444	2272	2462
				Wheat (grain and straw)			
1910	2850	3175	4200	3800	4325	4100	4375
1915	2280	2640	2840	2900	2940	2720	2820
1920	1040	2360	2620	2440	2800	2580	2620
Average	2057	2725	3220	3047	3355	3133	3272
				Timothy and clover (oats 1911)			
1911	2100	2100	2800	2400	2650	2500	3000
1916	3800	4660	5540	5800	6540	6060	6340
1921	1700	2860	3420	2760	3680	2840	3560
Average	2533	3207	3920	3653	4290	3800	4300
				Timothy and clover			
1912	1925	2200	2875	2250	3200	2175	3275
1917	2620	3520	3520	3640	3800	3500	4220
1922	1736	3824	4700	4984	5610	5310	6040
Average	2094	3181	3698	3625	4203	3662	4512
GENERAL AVERAGE	2545	3120	3721	3449	3928	3589	3960

application an average increase of a little over 1400 pounds per acre. The average increases for the calcium limestone treatments were slightly below those for the magnesian limestone.

The results obtained in this rotation give little basis for the use of as much as two tons of limestone, and indeed there may be some question as to the advisability of using more than one-half ton, especially where magnesian limestone is being applied.

The yield of nitrogen

Table 2 shows the yields of nitrogen for this rotation based on the dry weight of the crop and the nitrogen content of a representative sample. For a given crop, the yield of dry matter is, in most cases an index of the yield of nitrogen.

TABLE 2

Yield of nitrogen per acre in different crops in rotation

CROP	YEAR	NO LIME	½ TON		1 TON		2 TONS	
			Ca limestone	Mg limestone	Ca limestone	Mg limestone	Ca limestone	Mg limestone
First 5-year period, 1908–1912								
		lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
Corn	1908	38.5	42.2	56.1	47.2	51.5	49.8	52.4
Oats	1909	17.6	18.3	24.8	17.9	27.4	22.4	29.6
Wheat	1910	27.5	27.7	38.5	35.9	38.7	36.6	38.9
Oats	1911	30.1	28.5	40.0	32.3	38.9	34.0	43.0
Timothy and clover	1912	16.3	18.8	25.7	18.7	28.4	19.3	29.8
Average		26.0	27.1	37.0	30.4	37.0	32.4	38.7
Second 5-year period, 1913–1917								
Corn	1913	25.6	33.1	41.2	40.3	52.3	49.3	54.2
Oats	1914	19.7	17.0	21.5	22.2	23.4	20.8	24.0
Wheat	1915	24.1	28.3	30.5	31.5	29.6	28.7	29.7
Timothy and clover	1916	35.5	66.3	74.7	88.6	106.1	97.6	99.8
Timothy and clover	1917	19.9	28.5	27.8	30.9	32.3	29.7	34.6
Average		25.0	34.7	39.1	42.7	48.7	45.2	48.5
Third 5-year period 1918–1922								
Corn	1918	48.3	54.8	60.3	58.5	62.6	57.7	54.8
Oats	1919	24.4	34.4	35.7	35.8	42.1	36.0	41.5
Barley	1920	12.9	25.3	28.6	26.1	31.2	28.9	31.2
Timothy and clover	1921	14.9	22.3	26.2	23.9	30.6	24.5	28.4
Timothy and clover	1922	16.6	32.7	56.3	60.4	60.7	76.5	86.6
Average		23.4	33.9	41.4	41.0	45.4	44.7	48.5

With only a few exceptions the corn returned approximately 40 to 50 pounds of nitrogen per acre. The returns for the wheat and oats were considerably under these figures. The yields from the hay crop were very much influenced by the clover. In 1916 there was a second cutting most of which was clover

and the total yield of nitrogen was high. In 1917 there was only one cutting with a thin stand of clover and this was reflected in a low nitrogen yield. This was true also of the 1921 hay crop. In 1922 the second cutting of the hay accounted for a nitrogen yield of more than 60 pounds per acre for the 1- and 2-ton applications of limestone. In 1922 the plot that received two tons of magnesian limestone yielded more than five times as much nitrogen as the check plot. In 1916 the 1-ton application of magnesian limestone yielded three times as much nitrogen as the check. With but one exception the plots receiving 1-ton of magnesian limestone yielded more nitrogen than those receiving 2-tons of calcium limestone.

It is of interest to note the close agreement between the average yields for the second and third 5-year periods which range from about 25 pounds for the check plot to nearly 50 pounds for the 1- and 2-ton applications of magnesian limestone. In this connection it may be explained that there was very little clover on the check plot. The low average yield of nitrogen for the first 5-year period is due largely to the fact that in 1911 the timothy and clover were replaced by oats.

ROTATION 2 (PLOTS 28–34)

As in the case of rotation 1, the timothy and clover which was seeded in 1910 failed and was replaced by oats in the spring of 1911. The crop yields are shown in table 3. The lime treatments again showed some increase over the check plot in the majority of cases. The potatoes for 1909 (omitted from average), the rye for 1915 and the oats for 1911 were exceptions. The failure of the lime plots to show increases in 1911 was undoubtedly due to the failure of the timothy and clover and the substitution of oats, mention of which has already been made. The potato yields were somewhat irregular but in the majority of cases the lime-treated plots showed some increase over the check plot. The yields for this crop were all low; in 1909 it was less than one-fifth of the average for the other two years. The records fail to explain the reason of this failure.

An examination of the average yields for the five crops—15 years in all—shows that ½-ton of magnesian limestone caused an average increase of about 625 pounds of dry matter per acre over the check plot. One ton of magnesian limestone caused an average increase of only 124 pounds more than the ½-ton application; the 2-ton application caused an average increase of only 75 pounds more than the ½-ton application. In 10 years out of the 15, one ton of calcium limestone gave larger yields than an equal amount of magnesian limestone. In this rotation as in the previous one, the timothy and clover showed the greatest response to the lime. Taking the general averages the calcium limestone gave slightly higher yields than the magnesian limestone.

TABLE 3

Yield of crops in rotation 2 (acre basis)

YEAR	NO LIME	½ TON		1 TON		2 TONS	
		Ca limestone	Mg limestone	Ca limestone	Mg limestone	Ca limestone	Mg limestone
				Corn (grain and stover)			
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
1908	4800	6005	4745	5500	4423	5311	4961
1913	3475	4425	4700	4325	4750	4750	4725
1918	4883	5058	5358	5799	5294	5845	5696
Average	4386	5163	4934	5208	4822	5302	5127
				Potatoes			
1909*	1000	1020	1020	880	840	920	840
1914	6200	7040	5960	7100	5620	6560	5240
1919	5406	5615	6380	6695	6635	5760	6020
Average	5803	6328	6170	6898	6128	6160	5630
				Rye (grain and straw)			
1910	5425	6650	7150	5850	7500	6700	8050
1915	4080	3960	4040	4100	4320	3840	3880
1920	3920	4800	4760	5080	5000	5680	6040
Average	4475	5137	5317	5010	5607	5407	5990
				Timothy and clover (oats 1911)			
1911	2725	2850	2900	2650	2900	2700	2600
1916	4580	5280	5700	5980	6200	6100	5860
1921	2864	3348	2880	3570	3470	2840	3500
Average	3390	3826	3827	4067	4190	3880	3987
				Timothy and clover			
1912	2900	3650	3650	3425	3225	3800	3150
1917	3500	3680	3960	4440	3840	4500	3580
1922	3600	5360	5180	6800	6082	5680	5720
Average	3333	4230	4263	4888	4382	4660	4150
GENERAL AVERAGE	4277	4937	4902	5214	5026	5082	4977

*Omitted from average.

The yield of nitrogen

Table 4 shows the total yield of nitrogen per acre for the crops of this rotation and the averages for the three 5-year periods. These figures follow the crop yields with a fair degree of regularity although there are some exceptions. Without exception the 5-year averages show a larger yield of nitrogen for the

TABLE 4

Yield of nitrogen per acre in different crops in rotation 2

CROP	YEAR	NO LIME	½ TON		1 TON		2 TONS	
			Ca limestone	Mg limestone	Ca limestone	Mg limestone	Ca limestone	Mg limestone
First 5-year period, 1908–1912								
		lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
Corn	1908	44.0	63.3	50.3		42.6	55.5	47.7
Potatoes*	1909	3.7	3.6	3.6	3.3	3.2	3.4	3.4
Rye	1910	32.4	40.6	44.4	38.2	49.1	47.6	55.1
Oats	1911	35.7	41.2	39.7	38.2	41.8	36.0	37.8
Timothy and clover	1912	24.8	33.5	31.5	30.4	30.7	36.1	30.2
Average		34.2	44.7	41.5	35.6	41.1	43.8	42.7
Second 5-year period, 1913–1917								
Corn	1913	33.3	50.6	56.5	61.2	62.1	70.4	60.9
Potaotes	1914	22.5	23.8	21.7	20.9	21.1	20.3	19.3
Rye	1915	28.2	26.7	29.4	29.5	30.6	28.8	28.5
Timothy and clover	1916	53.9	61.7	79.2	82.7	90.8	101.9	96.6
Timothy and clover	1917	27.3	29.4	34.4	39.0	37.5	36.0	34.7
Average		33.0	38.5	44.2	46.7	48.4	51.5	48.0
Third 5-year period, 1918–1922								
Corn	1918	55.0	52.3	67.0	63.7	60.0	67.3	69.3
Potatoes	1919	16.9	23.8	27.9	26.8	20.3	23.3	25.2
Rye	1920	27.4	29.3	32.9	31.5	34.5	37.2	43.0
Timothy and clover	1921	24.8	31.2	31.4	44.5	47.8	35.2	51.1
Timothy and clover	1922	34.7	64.5	62.7	87.6	96.8	80.1	72.8
Average		31.8	40.2	44.4	50.8	51.9	48.6	52.3

* Omitted from average.

lime-treated plots than for the check plot. In the majority of cases the magnesian limestone gave slightly higher yields of nitrogen than the calcium limestone. It will be recalled from table 3 that the general averages for crop yields were slightly higher for the calcium limestone than for the magnesian limestone. This apparent discrepancy is undoubtedly due to the fact that in

a number of cases the percentage of nitrogen was higher in the dry matter from plots treated with magnesian limestone.

As in the case of rotation 1 the potato crop for 1909 was abnormally small and for this reason the nitrogen figures have here also been omitted from the average. In 1916 the timothy and clover yielded a second cutting which was reflected in the large amount of nitrogen recovered for that year. Only one cutting was taken in 1917 and this did not contain very much clover. The crop was small again in 1921 but in 1922 two cuttings were obtained. The yield of nitrogen for this year was 96.8 pounds per acre with one ton of magnesian limestone. The highest yield in 1916 was 101.9 pounds with two tons of calcium limestone. This is almost twice as much as was returned from the check plot this year. In the matter of nitrogen recovery for the 15-year period, one ton of limestone proved almost as effective as two tons.

ROTATION 3 (PLOTS 35–41)

The corn used here has been field corn and not sweet corn. It has been the practice to seed a green manure crop after the main crop. In most cases this green manure crop has been rye and vetch, or rye, vetch and clovers. These crops have aided materially in maintaining the supply of nitrogen and organic matter. No farm manure has been used. Somewhat more commercial nitrogen has been used for vegetable crops than for the general farm crops of rotations 1 and 2.

The yields for the crops of this rotation for the 15 years are shown in table 5. With only a few exceptions the lime-treated plots gave larger returns than the check plots. The lima beans and cucumbers showed the greatest response to the lime treatment. With two or three exceptions the potatoes also showed a good response but the yields were generally low. The 1909 crop, like that of rotation 2, was exceptionally small and the figures have been omitted from the averages.

The results for the tomatoes are somewhat irregular but distinct gains with the lime treatment are shown for 1910 and 1915. In 1920 the results are more irregular, the no-lime plot giving the highest yields.

Taking the 15-year averages, the 1- and 2-ton applications of magnesian limestone show somewhat higher yields than the same amounts of calcium limestone, but with the ½-ton application the order is reversed.

It is quite evident that the use of lime in this rotation has proved profitable, but it is also evident that the 2-ton application is excessive. Indeed, the general averages show that the ½-ton application has given almost as good yields as the 1-ton application and better than the 2-ton application. The results indicate that the 2-ton magnesian limestone treatment had a depressing effect on the lima beans in each of the three years and on the cucumbers in 1912 and 1922.

TABLE 5

Yield of crops in rotation 3 (acre basis)

YEAR	NO LIME	½ TON		1 TON		2 TONS	
		Ca limestone	Mg limestone	Ca limestone	Mg limestone	Ca limestone	Mg limestone
				Corn (grain and stover)			
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
1908	4792	4706	4537	4401	4953	5333	5206
1913	4125	4525	4750	4875	5375	5350	5400
1918	5242	5788	5435	6058	5820	5957	5915
Average	4720	5006	4907	5111	5383	5547	5507
				Potatoes			
1909*	640	600	740	720	880	760	690
1914	4800	6320	5680	6300	6260	5740	5100
1919	7165	10310	5425	9590	11445	7470	10355
Average	5982	8315	5552	7945	8852	6605	7727
				Tomatoes †			
1910	13455	21218	19043	19210	21688	17670	18973
1915	24080	28550	25454	27860	27720	25610	28924
1920	18728	15034	16664	13768	17747	9899	16352
Average	18754	21601	20387	20279	22385	17726	21416
				Lima beans (dry shelled)			
1911	540	800	740	750	750	715	620
1916	480	660	540	860	660	720	360
1921	380	620	1050	950	1330	1370	950
Average	467	693	777	853	913	935	643
				Cucumbers			
1912	5300	8000	7500	9300	5300	8800	4500
1917	8138	11618	13432	13536	13482	11688	13496
1922	5218	8948	9228	9496	9110	12726	8408
Average	6219	9522	10053	10777	9297	11071	8801
GENERAL AVERAGE	7228	9027	8335	8993	9366	8377	8819

* Omitted from average.

† These yields have been previously reported by Blair (1). In that paper the calculations for 1910 were, unfortunately, made on incomplete data. The figures given here are based on complete data.

The yield of nitrogen

The nitrogen returns in the different crops for the three 5-year periods for this rotation are shown in table 6. There is a fairly close agreement between

TABLE 6

Yield of nitrogen per acre in different crops in rotation 3

CROP	YEAR	NO LIME	½ TON		1 TON		2 TONS	
			Ca limestone	Mg limestone	Ca limestone	Mg limestone	Ca limestone	Mg limestone
First 5-year period, 1908–1912								
		lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
Corn	1908	41.5	45.5	42.7	44.9	49.8	50.7	53.5
Potaotes*	1909	2.4	2.3	2.9	2.9	3.4	3.2	2.8
Tomatoes†	1910	24.2	38.2	34.3	34.6	39.0	31.8	34.2
Lima beans	1911	19.5	28.0	26.3	28.1	27.7	25.8	24.6
Cucumbers‡	1912	5.8	8.8	8.3	10.2	5.8	9.7	5.0
Average		22.8	30.1	27.9	29.5	30.6	29.5	29.3
Second 5-year period, 1913–1917								
Corn	1913	43.0	49.1	46.5	53.4	56.3	55.4	62.1
Potatoes	1914	15.4	16.2	16.4	19.1	19.3	20.7	16.3
Tomatoes	1915	40.6	48.1	42.9	46.9	46.7	43.1	48.7
Lima beans	1916	16.7	22.4	19.3	29.4	23.2	22.8	14.3
Cucumbers	1917	9.5	14.8	16.9	18.3	16.9	15.0	15.9
Average		25.0	30.1	28.4	33.4	32.5	31.4	31.5
Third 5-year period, 1918–1922								
Corn	1918	58.5	68.7	69.9	72.0	70.0	72.0	72.9
Potatoes	1919	23.3	29.0	19.4	26.8	26.9	22.7	30.7
Tomatoes	1920	35.0	28.9	31.8	26.3	34.0	19.9	29.8
Lima beans	1921	12.6	19.8	36.6	31.9	43.3	49.2	37.0
Cucumbers	1922	5.5	9.4	8.9	10.4	9.7	13.1	8.6
Average		27.0	31.2	33.3	33.5	36.8	35.4	35.8

* Omitted from average.

† Per cent nitrogen not determined; estimated from 1915 and 1920 crops at 0.18 per cent.

‡ Per cent nitrogen not determined; estimated from 1922 crop at 0.11 per cent.

the average amount returned for the first and second 5-year periods. The average amount for the third 5-year period is slightly higher, due chiefly to the larger amount returned in the corn crop of 1918.

Of the crops in this rotation corn required the most nitrogen and the cucumbers the least. The 5-year averages showed an increase for the limed plots over the check plots. The lima beans for 1921 showed the largest increase, the 2-ton application of calcium limestone gave a yield of 49.2 pounds as against 12.6 pounds for the check. The corn, potatoes, tomatoes and cucumbers did not show large increases for the lime treatment, though in nearly all cases there was some increase. Taking the averages, the yields with the 1- and 2-ton applications were very nearly the same for the three periods and were only a little higher than the yields with the 1-ton application. By the same standard the calcium and magnesian limestone are about evenly balanced.

ROTATION 4 (PLOTS 42–48)

The yields of dry matter for this rotation are shown in table 7. This was originally planned as a forage crop rotation. With the exception of the year when corn comes in, two crops have been grown each year, one of which has been a legume. In some cases soybeans have been substituted for cowpeas, and in 1922 rye took the place of oats and peas. In most cases the corn did not show very large increases with the lime treatments. On the other hand fair increases were generally obtained where a legume crop appears. The winter vetch and cowpeas (or soybeans) show marked response to the lime. For the corn, oats and peas and millet, the 1- and 2-ton applications of limestone have not greatly increased the yields over the ½-ton application.

With two or three exceptions, notably the 2-ton magnesian-limestone treatment, the corn showed fair gains for the lime treatments. For the other crops of the rotation distinct increases are noted in the majority of cases. For example, in 1921 the rye and cowpeas on the no-lime plot yielded 5070 pounds of hay, whereas with 1 ton of calcium limestone the yield was 8208 pounds and with 1 ton of magnesian limestone, 9600 pounds. Again in 1922 the oats and peas and cowpeas on the no-lime plot yielded 3920 pounds while with one ton of both forms of limestone the yield was over 6000 pounds of hay.

In most cases the 2-ton application of calcium limestone gave larger yields than one ton of either, and in 12 cases out of 15 it gave larger yields than the two tons of magnesian limestone.

The 15-year averages show a slight advantage in favor of the magnesian limestone when used in ½- and 1-ton amounts, but with the 2-ton application there is some indication of injury.

The results taken as a whole indicate that magnesian limestone favors nitrogen fixation rather more than does calcium limestone, although as already pointed out there were a few cases where the 2-ton application undoubtedly resulted in some injury to the crop.

TABLE 7

Crop yields in rotation 4 (acre basis)

YEAR	NO LIME	½ TON		1 TON		2 TONS	
		Ca limestone	Mg limestone	Ca limestone	Mg limestone	Ca limestone	Mg limestone
			Corn (grain and stover)				
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
1908	4218	4232	5203	4645	4942	5345	3827
1913	4025	4600	3975	4825	4525	5325	4200
1918	5025	5484	5340	5365	5030	5723	5054
Average	4423	4772	4839	4945	4832	5464	4360
			Oats and peas—millet				
1909	3136	3240	3689	2750	3797	3322	3659
1914	1920	2680	2260	2820	2360	2960	2220
1919	2760	4680	4040	4800	4000	4920	3920
Average	2605	3533	3330	3457	3386	3734	3266
			Winter vetch and rape				
1910	2900	4300	4900	5250	3350	5650	5650
1915	1920	2680	3840	3520	4960	4280	5420
1920	4200	4008	3600	3372	4820	4560	4580
Average	3007	3663	4113	4047	4377	4830	5217
			Rye and cowpeas				
1911	4950	5500	4700	5850	5000	5550	5100
1916	4180	4900	5240	5320	5740	6000	4780
1921	5070	7560	8880	8208	9600	9172	8620
Average	4733	5987	6273	6459	6780	6907	6167
			Oats and peas—cowpeas				
1912	3050	3675	3425	3900	3475	3500	3475
1917	3280	3840	4480	4420	4420	4720	4420
1922	3920	5920	6280	6080	6480	7280	6400
Average	3417	4478	4728	4800	4792	5167	4765
GENERAL AVERAGE	3637	4486	4656	4741	4833	5220	4755

The yield of nitrogen

This rotation has yielded much more nitrogen than any of the three preceding rotations. This reflects, in a striking manner, the influence of the legumes. As shown in table 8, the yields for the vetch and rape in 1910 ranged from 66.6 pounds for the check plot to 137.1 and 147.7 pounds, respectively,

TABLE 8

Yield of nitrogen per acre in different crops in rotation 4

CROP	YEAR	NO LIME	½ TON		1 TON		2 TONS	
			Ca limestone	Mg limestone	Ca limestone	Mg limestone	Ca limestone	Mg limestone
First 5-year period, 1908–1912								
		lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
Corn	1908	37.6	39.8	47.9	42.0	46.2	53.9	37.7
Oats and peas, millet	1909	54.1	57.1	68.2	50.6	71.3	59.4	61.0
Rape, vetch	1910	66.6	90.7	125.6	129.8	136.6	137.1	147.7
Rye, cowpeas	1911	90.4	106.2	77.9	116.6	82.8	98.6	87.5
Oats and peas, cowpeas	1912	64.5	90.1	75.8	103.0	87.3	84.1	90.6
Average		62.6	76.8	79.1	88.4	84.8	86.6	84.9
Second 5-year period, 1913–1917								
Corn	1913	37.0	45.3	39.8	52.1	43.0	55.4	44.8
Oats and peas, millet	1914	29.9	47.5	40.1	50.4	39.4	54.1	42.9
Vetch, rape	1915	23.8	47.1	79.3	71.7	109.8	90.2	116.7
Rye, cowpeas	1916	52.0	57.8	85.7	69.5	95.9	95.8	78.0
Oats and peas, cowpeas	1917	59.7	77.4	104.8	95.7	104.9	121.0	119.4
Average		40.5	55.0	69.9	67.9	78.6	83.3	80.3
Third 5-year period, 1918–1922								
Corn	1918	59.4	61.6	63.0	56.6	58.3	66.7	56.5
Oats and peas, millet	1919	36.5	62.5	55.1	70.6	54.7	72.0	57.1
Rye and vetch, rape	1920	32.9	45.6	47.8	41.1	74.2	55.2	69.6
Rye and vetch, soybeans	1921	61.9	120.1	156.6	175.7	145.8	163.7	172.5
Rye and soybeans	1922	50.9	82.0	87.6	84.6	115.1	120.7	117.3
Average		48.1	74.4	82.0	85.7	89.6	95.7	94.6

for the 2-ton applications of the calcium and magnesian limestone. In 1915 the same crops gave a yield ranging from 23.8 pounds for the check plot to 116.7 pounds for the 2-ton application of magnesian limestone. In 1920 when rye was seeded with the vetch the yield of nitrogen was not so high. The highest yields for the entire period were obtained with rye and vetch, and soybeans in 1921. In this case the check plot yielded 61.9 pounds and the largest yield was 175.7 pounds with one ton of calcium limestone.

With very few exceptions the yield was less on the check plot than on the lime treated plots. In the majority of cases the 1-ton application yielded more than the ½-ton application. Judged by the 5-year averages, the 2-ton application shows very little advantage over the one ton. By the same standard the magnesian limestone shows a slight advantage over the calcium limestone in five out of nine chances.

The applications of commercial nitrogen for this rotation have been very light and it is therefore evident that the legume crops have drawn their supply of nitrogen largely from the atmosphere, otherwise the yields could not have been maintained as they have for a period of 15 years. Furthermore the nitrogen determinations on samples of soil from these plots indicate that the soil is not losing in its content of nitrogen.

INFLUENCE OF LIME ON THE PERCENTAGE OF NITROGEN IN THE CROP

It is well known that under certain conditions the nitrogen content of plants may be considerably modified. An increase in the amount of readily available nitrogen frequently results in an increase in the percentage of nitrogen in the crop.

In pot experiments with sand cultures it has been possible to increase the percentage of nitrogen in the dry matter from about 1 per cent under normal conditions to nearly 3½ per cent, by the use of large amounts of nitrate of soda (6). Corn and other non-legume crops grown after a legume cover crop will frequently show a higher percentage of nitrogen in the dry matter than when the same crop is grown after a non-legume green manure crop.

On the other hand nitrogen starvation may also result in a plant with a per cent of nitrogen higher than normal. It has been shown that in the majority of cases, legume crops grown on limed land, recover a larger amount of nitrogen per acre than the same crops grown on unlimed acid land. This may be due to an increase in the yield of dry matter or to a higher percentage of nitrogen in the dry matter, or both.

Apparently the cause of the higher percentage of nitrogen in the dry matter from limed plots, as contrasted with that from unlimed plots, has not been fully investigated. It may be due in part to the fact that frequently there is a greater abundance of weeds and grasses on the unlimed than on the limed plots. In this connection Hall (2) says: "Again on examining the composition of the herbage, it was seen that on the same three plots which gave an increase of crop, the lime has brought about a great increase in the proportion of leguminous plants. On plot 6 it has risen from 11 to 20 per cent, on plot 7 from 22 to 42 per cent and on plot 15 from 3 to 35 per cent." Hall believes that the development of the legume plant is largely dependent on a supply of potash, and further points out that these three plots have received potash every year for some time and consequently there was a large reserve of potash in the soil. The lime he believes, released this potash and therefore acted as an

application of potash. In other words, according to Hall's view, the lime acts only where there is a residue of potash.

Hall's line of reasoning is not entirely borne out by the work at the New Jersey Station. Here even with liberal annual applications of soluble potash, volunteer legumes do not come in to any extent if lime is withheld. On the other hand when lime is applied under these conditions, it is practically impossible to prevent the coming in of volunteer legumes, the clovers especially. It would seem that the differences noted may be reconciled by the fact that the Rothamsted soils are well supplied with calcium carbonate.

It would appear, that the percentage of nitrogen in the dry matter may be very much influenced by the amount of available nitrogen at the disposal of the growing crop. In the case of the higher percentage of nitrogen from limed plots where the crop is strictly a legume, as in the case of soybeans grown for seed, the lime undoubtedly favors those organisms which aid the plant in getting nitrogen from the air, and the plant thus assisted, becomes a more efficient nitrogen accumulator. In confirmation of this view, counts have shown far more root nodules on soybean plants from limed than from unlimed plots (5); likewise the plants from the limed plots were much larger and healthier in appearance than those from the unlimed plots. This could account for the higher percentage of nitrogen in the immediate crop and might also mean more available nitrogen for the succeeding crop whether it be a legume or a non-legume.

Nitrogen determinations have been made for practically all of the crops in the four rotations for the 15-year period. These data are given in tables 9 and 10 for the crops of rotations 1 and 4 which are fairly representative. They have been further shortened by averaging the figures for the three amounts of limestone in each case; i.e., the calcium limestone is represented by averaging the figures for the $\frac{1}{2}$-, 1-, and 2-ton applications and the figures representing the magnesian limestone were obtained in a similar manner.

A study of these two tables will show that in the majority of cases crops from the limed plots showed a higher percentage of nitrogen than those from the unlimed plots. A closer examination however, shows that the legume crops are largely responsible for throwing the balance in favor of the lime treated plots. In the case of the non-legume crops alone, the figures are frequently reversed. The legume crops on the other hand frequently show a striking increase in percentage of nitrogen for the limed plots over the unlimed plots. For example, cowpeas in 1916 in rotation 4 showed 1.47 per cent nitrogen with no lime and 2.33 per cent with magnesian limestone; the same crop in 1922 shows 1.51 per cent nitrogen with no lime and 2.48 per cent for the magnesian limestone, the difference being nearly 1 per cent in each case.

For a given crop the percentage of nitrogen does not vary greatly for the two forms of limestone, but in the majority of cases there is a slight difference in favor of the magnesian limestone. This is shown by the figures in the right hand column of the tables.

TABLE 9

Nitrogen in the crops of rotation 1

LIME TREATMENT		CORN—FIRST YEAR		OATS—SECOND YEAR		WHEAT—THIRD YEAR		TIMOTHY AND CLOVER—FOURTH YEAR		TIMOTHY AND CLOVER—FIFTH YEAR	
		Grain	Stover	Grain	Straw	Grain	Straw	1st cutting	2d cutting	1st cutting	2d cutting
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*				
No lime	1908–1912	1.31	0.65	2.20	0.86	1.91	0.45	2.12†	0.97‡	0.85	
	1913–1917	1.213	0.573	2.024	0.731	1.968	0.295	0.866	2.161	0.760	
	1918–1922	1.469	0.712	2.224	0.526	1.712*	0.870*	0.874		0.945	1.074
Ca limestone	1908–1912	1.32	0.77	2.26	0.85	1.78	0.44	2.14†	0.82‡	0.86	
	1913–1917	1.335	0.744	2.196	0.808	2.003	0.311	1.174	2.596	0.836	
	1918–1922	1.488	0.789	2.455	0.773	1.732*	0.538*	0.837		1.126	1.700
Mg limestone	1908–1912	1.37	0.85	2.33	0.92	1.84	0.43	2.26†	0.91‡	0.90	
	1913–1917	1.377	0.770	2.246	0.844	1.994	0.321	1.122	2.648	0.819	
	1918–1920	1.541	0.848	2.443	0.882	1.792*	0.605*	0.798		1.117	1.544

* Barley. † Oats (grain). ‡ Oats (straw).

TABLE 10

Nitrogen in the crops of rotation 4

LIME TREATMENT		CORN—FIRST YEAR		SECOND YEAR		THIRD YEAR		FOURTH YEAR		FIFTH YEAR	
		Grain	Stover	Oats and peas	Millet	Vetch	Rape	Rye	Cowpeas	Oats and peas	Cowpeas
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
No lime	1908–1912	1.30	0.64	2.01	1.28	3.11	1.54	1.47	2.33	1.98	2.35
	1913–1917	1.361	0.721	1.658	1.186	3.216	0.981	1.049	1.470	1.293	2.785
	1918–1922	1.544	0.906	1.386	1.160	0.756*	0.818	1.141*	1.288†	1.053‡	1.506
Ca limestone	1908–1912	1.40	0.72	2.26	1.26	3.12	1.51	1.42	2.72	2.29	2.83
	1913–1917	1.420	0.837	1.893	1.379	3.335	1.064	1.095	1.653	1.823	2.968
	1918–1922	1.423	0.916	1.415	1.462	1.185*	1.216	1.850*	1.818†	0.718‡	2.313
Mg limestone	1908–1912	1.44	0.72	2.38	1.11	3.39	1.49	1.23	2.51	2.23	2.84
	1913–1917	1.414	0.794	1.841	1.432	2.916	1.133	1.020	2.329	2.054	3.274
	1918–1922	1.500	0.917	1.402	1.379	1.516*	1.277	2.038*	1.457†	0.849‡	2.484

* Rye and vetch. † Soybeans. ‡ Rye.

TABLE 11

Lime requirement and nitrogen content of all soils at the close of each period (and pH values for 1922)

PLOT NUMBER	TREATMENT PER ACRE	LIME (CaO) REQUIREMENT PER 2,000,000 POUNDS OF SOIL			NITROGEN CONTENT				REACTION 1922
		1913*	1918†	1922‡	1913	1918	1922		
								Soil 1	
	Rotation 1.—General farm crops								
	tons	*lbs.*	*lbs.*	*lbs.*	*per cent*	*per cent*	*per cent*		*pH*
21	No lime	1200	1200	1500	0.077	0.0794	0.082	0.059	5.2
	Ca limestone:								
22	0.5	1000	600	800	0.075	0.0798	0.082	0.052	6.3
23	1.0	600	400	400	0.075	0.0791	0.084	0.051	6.4
24	2.0	000	*Alk.*	*Alk.*	0.081	0.0901	0.092	0.061	6.8
	Mg limestone:								
25	0.5	600	400	1000	0.091	0.0959	0.095	0.062	5.7
26	1.0	700	400	600	0.093	0.1015	0.108	0.074	6.4
27	2.0	000	*Alk.*	*Alk.*	0.092	0.1010	0.108	0.071	7.0
	Rotation 2.—General farm crops and potatoes								
28	No lime	800	1400	1400	0.094	0.0994	0.102	0.067	5.6
	Ca limestone:								
29	0.5	800	1000	900	0.097	0.1019	0.101	0.065	5.6
30	1.0	600	400	300	0.094	0.0931	0.097	0.059	6.4
31	2.0	100	*Alk.*	100	0.098	0.1041	0.101	0.058	6.8
	Mg limestone:								
32	0.5	700	400	600	0.093	0.1025	0.100	0.058	6.4
33	1.0	400	*Alk.*	300	0.091	0.0954	0.097	0.054	6.7
34	2.0	300	200	100	0.089	0.0966	0.100	0.049	6.9
	Rotation 3.—Corn, potatoes, market garden crops								
35	No lime	1100	1200	1200	0.093	0.0921	0.093	0.062	5.4
	Ca limestone:								
36	0.5	800	1000	900	0.094	0.0912	0.093	0.059	5.5
37	1 0	600	800	600	0.081	0.0837	0.087	0.055	6.3
38	2.0	400	*Alk.*	300	0.075	0.0808	0.083	0.055	6.8
	Mg limestone:								
39	0.5	1100	1000	600	0.077	0.0800	0.080	0.050	6.2
40	1.0	700	400	600	0.080	0.0837	0.082	0.060	6.4
41	2.0	500	400	200	0.082	0.0800	0.081	0.059	6.9

* Limestone applied in spring 1908.
† Limestone applied in spring 1913.
‡ Limestone applied in spring 1918.

TABLE 11—*Continued*

PLOT NUMBER	TREATMENT PER ACRE	LIME (CaO) REQUIREMENT PER 2,000,000 POUNDS OF SOIL			NITROGEN CONTENT				REACTION 1922
		1913*	1918‡	1922‡	1913	1918	1922		
								Soil 1	
	Rotation 4.—Forage crops								
	tons	*lbs.*	*lbs.*	*lbs.*	*per cent*	*per cent*	*per cent*		*pH*
42	No lime	1200	1400	1200	0.084	0.0899	0.090	0.046	5.2
	Ca limestone:								
43	0.5	1100	1200	1000	0.095	0.0998	0.101	0.060	5.5
44	1.0	700	800	600	0.099	0.1012	0.102	0.056	6.3
45	2.0	600	400	200	0.089	0.0864	0.084	0.040	6.7
	Mg limestone:								
46	0.5	1100	1200	600	0.079	0.0773	0.077	0.053	6.3
47	1.0	500	400	400	0.067	0.0679	0.069	0.035	6.4
48	2.0	300	*Alk.*	200	0.098§	0.0652	0.063	0.033	6.8
AVERAGE					0.087	0.0893	0.091		

§ Probably an error in determination; 1914 sample gives 0.070 per cent N.

The rye and vetch crop in 1920 in rotation 4 supplies a good illustration of the value of lime when a legume crop is combined with a non-legume. Here the no-lime plot where the vetch did poorly showed 0.756 per cent nitrogen while the plots that received magnesian limestone showed a percentage just twice as great. Besides a large increase in dry matter already referred to, the lime-treated plots yielded a feeding material much richer in protein than the unlimed.

THE INFLUENCE OF THE LIME ON THE LIME REQUIREMENT AND NITROGEN CONTENT OF THE SOIL

All of these soils have been analyzed for lime requirement and total nitrogen at the close of each 5-year period. The figures for the first two periods have been published (3, 4). They are given here again for comparison with the data for the third 5-year period (table 11). The hydrogen-ion concentration in terms of pH are reported for the 1922 samples.

In collecting samples, a number of borings were taken from each plot to a depth of $6\frac{2}{3}$ inches and from these a composite sample was made. The portion of the dry sample not passing a 2-mm. sieve was discarded. Nitrogen determinations were made by the Kjeldahl method and lime requirement by the Veitch method.

Lime requirement

The figures for lime requirement show a general decrease with increase of lime application, the 2-ton application leaving the soil alkaline or nearly so after 5 years. With one exception the no-lime plots showed but little change since the first sampling in 1913 and the requirement ranged between 1000 and 1500 pounds CaO per acre. With a few exceptions, the plots which have received one-half ton of limestone per acre showed a requirement close to 1000 pounds and those that have received one ton per acre, a requirement ranging in most cases between 300 and 600 pounds.

The magnesian limestone appears to have a slight advantage in the matter of satisfying the lime requirement.

The hydrogen-ion determinations are in general accord with the lime requirement determinations. For example, the four no-lime plots showed a range of pH 5.2 to pH 5.6 and the plots which received two tons of limestone per acre a range of pH 6.7 to pH 7.0. From these figures it would appear that in many cases at least, the pH values might be used as a measure of the lime requirement.

Percentage of nitrogen in the soil

Figures showing percentage of nitrogen in the soil are quite consistent for the three periods. It is gratifying indeed to find that the figures are in most cases slightly higher for the third period than for the two preceding periods. This is confirmatory evidence that under the different systems of cropping the soil is not being depleted of its stores of nitrogen and organic matter. Furthermore, this result is being accomplished without the use of any farm manure and, excepting the market garden rotation, with a minimum of commercial nitrogen.

It would appear from the percentage of nitrogen shown for plots 21, 22, 23, and 46, 47, 48, that the soil of these plots is naturally not so good as the soil constituting the remainder of the plots. The percentages of nitrogen in the subsoil seem to confirm this view. Taking all rotations for the entire period there is a slight increase in the percentage of nitrogen as shown by the general average for 1922, in comparison with the averages at the two preceding sampling periods. It is highly probable that these soils would have decreased in percentage of nitrogen rather than increased, had legume crops been entirely omitted from the rotations.

Taking the results for all of the rotations, it may be said that there is no definite indication that the lime has tended to cause an unusually rapid disappearance of the nitrogen from the soil.

SUMMARY

1. An experiment involving the use of magnesian and non-magnesian limestone, in three different amounts, as compared with a check plot has been carried out for a period of 15 years, in connection with four different 5-year crop rotations.

2. The soil is a Sassafras loam, gravelly phase, and previous to the experiment had not been limed for many years.

3. In each of the four rotations legume crops were introduced to some extent, either as one of the main crops or as a green manure crop between the main crops.

4. Mineral fertilizers (acid phosphate and muriate of potash), have been used at the rate of 300–400 pounds per acre for the former and 100–200 pounds for the latter. Commercial nitrogenous fertilizers in amounts equivalent to 160–200 pounds per acre of nitrate of soda have been used. No farm manure has been used during the entire 15 years.

5. With few exceptions the lime-treated plots have shown substantial increases in crop yield over the check plots. Of the crops used in the various rotations the legumes have shown a greater response to the lime treatments than the non-legumes, though the latter have usually shown some response.

6. In most cases the 1-ton application has given some increase over the ½-ton application but, in a number of cases at least, this increase is not sufficient to justify the additional expense. In the majority of cases the 2-ton application gave but slight increase in yield over the 1-ton application thus indicating that the 2-ton application is excessive from the standpoint of economy.

7. In a few cases there was indication of crop injury from the use of 4000 pounds of magnesian limestone per acre. Aside from this the two forms of limestone gave results that are quite similar, though taking the records for the entire period, there is a slight difference in favor of the magnesian limestone.

8. In the matter of the amount of nitrogen recovered from the crop, the difference between the unlimed and limed plots is more striking than the differences in the case of the crop yields, but here as with the crop yields, the 2-ton application gave very little increase over the 1-ton application.

In this case, also, the magnesian limestone seemed to show a slight advantage over the other form.

9. For the legume crops especially, the limed plots showed a higher percentage of nitrogen in the dry matter than the non-legume crops. In some cases the difference was very pronounced. This clearly indicates an improvement in the quality of the crop as well as an increase in the quantity.

10. In the case of mixed crops, such as mixed hay, it is suggested that this increase in percentage of nitrogen may have been due in part to the fact that the lime stimulates the growth of legume plants to the detriment of non-legumes.

In the case of strictly legume crops it is suggested that the increase in nitrogen content may have been due to the stimulating effect of the lime on the organisms which aid the plant in getting nitrogen from the air.

11. There is evidence that the magnesian limestone favors nitrogen fixation rather more than calcium limestone.

12. With the increase in the amount of lime applied there is a decrease in the lime requirement of the soil as determined by the Veitch method. The

2-ton application left the soil near the neutral point at the end of each 5-year period. With one ton the requirement is in most cases between 300 and 600 pounds and with one-half ton about 600 to 1200 pounds per acre. In most cases the check plots indicate a requirement of about 1200 to 1500 pounds per acre.

13. The hydrogen-ion concentration decreased gradually as the lime applications were increased, and the work indicates that this method may, in some circumstances, be used for the determination of the lime requirement.

14. The nitrogen content of the soil has remained fairly constant during the last 10 years of the period with a slight upward tendency. This stands as evidence that under the systems of cropping practiced in this experiment the soil is not being depleted of nitrogen and organic matter. Neither are the yields decreasing. Undoubtedly the legume crops have been a factor in maintaining the fertility of the soil.

15. In general there is no definite indication that lime has tended to cause an abnormally rapid disappearance of nitrogen and organic matter from the soil.

16. The results of this experiment indicate that it is not necessary to fully satisfy the lime requirement of the soil as commonly expressed in order to get good results with most farm crops.

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